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INFLUENCE OF CULTURE CONDITIONS UPON THE GROWTH AND DEVELOPMENT OF DICTYOSTELIUM DISCOIDEUM[1]

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INTRODUCTION

Dictyostelium discoideum, a new species of the Acrasieae described by the writer (*18*),[3] was recently shown to be capable of feeding upon bacteria belonging to many different species when these were grown upon hay-infusion agar (*19*). This medium was accordingly considered admirable for investigating the "host range" of a species of the Dictyosteliaceae. Nevertheless, it left much to be desired and certain of its characters were plainly objectionable: First, it was not an optimum medium for any bacterial species studied and consequently the growth of the *Dictyostelium* was regularly limited by the restricted growth of the host bacteria; second, it was of unknown and somewhat variable composition despite all possible care in the selection of source material and in the preparation [illegible] ird, it was light brown in color and, because [illegible] itting light through it, was not well suited for [illegible] of myxamoebae in situ on agar plates.

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With these objection [illegible] ertook to find a medium or media that would b [illegible] is limitations of hay-infusion agar, and, at the same time, endeavored to study critically the combined influence of the culture medium and of the associated bacteria upon the growth and subsequent development of a species of the Dictyosteliaceae. The need for such a study was apparent. Except for the work of Potts (*17*) there has been little appreciation of the changes brought about in culture media by the growth of bacteria associated with these slime molds; and no careful effort has been made to analyze the influence of such changes on the behavior of an organism of this group. Further, species of the Dictyosteliaceae have been found to occur commonly in soil and decaying vegetation (*20*), but surprisingly little is known of the environmental conditions that favor or retard their growth in these situations.

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[2] The writer wishes to acknowledge his indebtedness to Prof. William H Weston, Jr , of Harvard University, under whose direction the present study was pursued, for valuable counsel and criticism during the progress of the work, to Prof Kenneth Thimann, likewise of Harvard University, for advice in connection with the studies of hydrogen-ion concentration; and to Dr. Charles Thom and N R Smith, of the Division of Soil Microbiology, Bureau of Plant Industry, U S Department of Agriculture, for their generous cooperation in making this investigation possible

[3] Italic numbers in parentheses refer to Literature Cited, p 197.

Because of its unique developmental behavior (*18*) and because its growth with different bacterial associates can be conveniently measured in terms of the number and size of the sorocarps produced, *Dictyostelium discoideum* is especially adapted for a study of the interrelationships between an amoeboid organism and the bacteria on which it feeds (*19*). For the present study this species was selected because, in its growth and more particularly in the development of its fruiting structures, *D. discoideum* is extremely sensitive to conditions of culture and slight changes in environment are reflected in the pattern of the sorocarps developed. Thus the favorableness of cultures to this slime mold can be measured to a degree that is not practicable with other species of this group, or, for that matter, with soil amoebae generally.

REVIEW OF LITERATURE

Students of the Dictyosteliaceae have regularly cultivated these organisms in the laboratory, and in their studies a wide variety of media have been employed. Brefeld used cooked horse dung as a solid substratum for mass cultures and a filtered dung decoction for microcultures in his pioneer work on *Dictyostelium mucoroides* Bref (*3*), and later in his study of *Polysphondylium violaceum* Bref. (*4*). Van Tieghem employed cakes of beer yeast and drops of fresh urine for cultivating *Acrasis granulata* Van Tieghem and species of *Dictyostelium* (*25*), and later he used decaying broadbeans and urine for culturing *Coenonia denticulata* Van Tieghem (*26*). Olive (*14*), in his comprehensive study of the Acrasieae, found weak horse-dung decoction and peptone-solution agars to be the most satisfactory of the various media tested. Harper (*9, 10*) likewise utilized a weak horse-dung decoction both as a liquid and as an agar medium for his morphogenetic studies of *Dictyostelium mucoroides* (*8*) and *Polysphondylium violaceum*. Raper and Thom (*20*) cultivated species of the Dictyosteliaceae upon hay, dung, and carrot-infusion agars and upon mannite agar to which rat dung was added. Similarly, the writer (*18*) cultivated *Dictyostelium discoideum* upon hay- and dung-infusion agar in his initial study of this species.

In all the above investigations bacteria were reported as being present in the cultures, but in no case was the identity of the accompanying bacteria known, nor was any role of importance ascribed to them. On the other hand, some investigations have been made in which the identity of the associated bacteria was known and in which a role of primary importance was attributed to them, either directly as a source of food for the slime molds (*13, 15, 16, 17, 19, 21, 22, 24, 27*) or indirectly as agents capable of altering the culture medium (*12*).

The possibility of the accompanying bacteria changing the culture medium was first recognized by Nadson (*12*), who cultivated *Dictyostelium mucoroides* upon sterilized dung, and upon gelatin and agar media containing malt, an extract of dung, or peptone and inorganic salts. He reported that *Bacillus fluorescens liquefaciens* was the habitual associate of *D. mucoroides*, and that in cultures with this bacterium the slime mold developed with an intensity and vigor equal to that under natural conditions. This led him to conclude that the bacteria favored the growth of *Dictyostelium* by creating an alkaline medium through the production of ammonia.

Potts (*17*) cultivated *Dictyostelium mucoroides* upon sterilized dung and dung infusion, upon infusion agar made from the stems of *Vicia faba*, and upon an extract of corn (maize) grains which he employed either as a liquid or solid medium. Employing corn gelatin, which he found to be especially favorable, he observed that *Dictyostelium* developed only in the presence of bacteria, and he succeeded in isolating the slime mold with a single bacterial species, which he described as *Bacterium fimbriatum*. Using this association, he further studied the relationship existing between these organisms by cultivating them upon a large number of synthetic media, each of which contained some inorganic or organic source of nitrogen together with some sugar, organic acid, or other source of carbon. While he obviously misinterpreted the manner in which the slime mold fed upon the accompanying bacteria, believing they were digested extracellularly, he made no mistake in his conclusion that the *Dictyostelium* was dependent upon bacteria for nutriment. Furthermore, he was not unmindful of the influence the bacteria might exert through altering the culture medium, and reported that *D. mucoroides* could grow in slightly acid to strongly alkaline media but grew best in media of a mild alkaline reaction.

Employing a maltose peptone agar medium, Vuillemin (*27*) cultivated *Dictyostelium mucoroides* in association with *Bacillus fluorescens non-liquefaciens*. In the same year Pinoy (*15*) succeeded in growing *D. mucoroides* in association with *B. fluorescens liquefaciens*, *B. coli communis*, and *Microbacillus prodigiosus* upon pieces of sterilized carrot. In a more comprehensive study 4 years later, in which *D. purpureum* Olive and *Polysphondylium violaceum* as well as *D. mucoroides* were studied, and in which five additional bacterial associates for *D. mucoroides* were reported, Pinoy (*16*) employed for the most part a flaxseed-infusion agar. Again he profitably used strips of carrot, but found potato unsuitable, although it afforded a favorable medium for *B. fluorescens liquefaciens*. In addition, he reported that agar containing bouillon or peptone would not support the growth of *D. mucoroides*, although ample bacteria were present, unless lactose, maltose, or glucose was added. He apparently did not consider the possible influence of acidity or alkalinity upon the growth of the slime mold.

Skupienski (*23*, *24*) employed a weak hay infusion either as a liquid medium or incorporated in agar, and, like Nadson (*12*) and Pinoy (*16*), cultivated *Dictyostelium mucoroides* with *Bacillus fluorescens liquefaciens*. Oehler (*13*) cultivated the same slime mold upon nonnutrient agar; and while he did not report the identity of any associated bacteria, he implied that the *Dictyostelium* was grown with a number of different species. Obtaining his culture from Oehler, Schuckmann (*21*, *22*) subsequently cultured *D. mucoroides* upon dilute beef-bouillon agar in association with *B. coli communis* and two additional unidentified Gram-negative bacteria.

Hay-infusion agar alone was employed by the writer (*19*) in earlier work dealing with the growth and development of *Dictyostelium discoideum* in association with a large number of different bacterial species.

INFLUENCE OF CULTURE MEDIUM AND OF BACTERIAL ASSOCIATE

PRELIMINARY STUDIES

A number of substrata were investigated that were more or less comparable with those employed and recommended by earlier students of the Dictyosteliaceae. Such media included horse-dung-infusion agar (*3, 4, 8, 9, 10, 12, 14, 17, 21*), carrot-infusion agar (*15, 20*), corn-meal agar (*17*), flaxseed-infusion agar (*16*), bean-infusion agar (*26*), yeast agar (*25*), nonnutrient agar (*13*), and nutrient-broth agar (*21, 22*). None of these media proved wholly suitable, however, and each was unsatisfactory from one or more points of view. Upon dung, corn meal, flaxseed, and nonnutrient agars *Pseudomonas fluorescens* Migula [4] and *Serratia marcescens* Bizio, the species selected as bacterial associates for these preliminary studies, failed to grow as luxuriantly as desired; consequently, the growth of *Dictyostelium discoideum* was limited accordingly. Upon bean, yeast, and nutrient-broth agars the bacteria grew satisfactorily; but *D. discoideum* grew very poorly upon bean agar and failed completely to grow upon yeast and nutrient-broth media. Carrot agar alone proved reasonably satisfactory, for it supported a good growth of bacteria and subsequently of *Dictyostelium*. However, it could not be considered an especially favorable medium because, like hay-infusion agar, it was of unknown and, to a limited degree, of variable composition.

STUDIES WITH ENDO'S AND ALLIED MEDIA

Having failed to secure an entirely satisfactory culture medium among the above-named substrata, attention was next directed toward agar media in which the essential components were peptone and some type of sugar. The possibilities inherent in this combination of nutrients were first suggested to the writer by observing a culture of *Escherichia coli* (Migula) Castellani and Chamlers growing upon Endo's medium.[5] The bacteria grew well upon this medium, and from the writer's earlier studies with hay-infusion agar (*19*) *E. coli* was known to afford excellent food for *Dictyostelium discoideum*. Endo's medium was, therefore, secured for the purpose of determining whether the slime mold could grow upon it in association with *E. coli*.

The growth of *Dictyostelium discoideum* was immediate and excellent. Pseudoplasmodia and sorocarps began developing on the second day after the spores were introduced and continued to form for several days thereafter. At the end of a week the bacteria were almost wholly consumed and the growth of *Dictyostelium* had virtually ceased. Sorocarps were very abundant and were entirely normal in shape and proportions, the basal disks being circular, flat, and expanded; the sorophores were straight or slightly sinuous and evenly tapered; and the sori were large, yellow in color, and spherical to slightly elliptical in form. (See fig. 3.)

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[4] In discussing the writer's work, the nomenclature for the bacterial species studied is that of Bergey (*2*), but in reviewing the work of earlier investigators, the nomenclature used by those investigators is followed.

[5] Endo's agar is almost universally used for differentiating between *Eberthella typhosa* (Zopf) Weldin (*Bacillus typhosus* Zopf) and *Escherichia coli* (*Bacillus coli communis* Steinberg). The essential ingredients of this medium are lactose and peptone as nutrient materials, fuchsin decolorized with sodium sulphite as an indicator, and usually sodium carbonate or dipotassium phosphate to render the medium alkaline. When *E. coli* is grown upon this medium the lactose is broken down with the consequent production of aldehydes, and hence the bacterial colonies and the medium underlying them become deep red in color.

Since a medium of known[6] and easily reproducible composition had been found upon which *Dictyostelium discoideum* grew luxuriantly and developed normally in association with *Escherichia coli*, it now became desirable to make a thorough study of this medium and to determine just what components were essential to obtain a good growth of *E. coli* and subsequently of *D. discoideum*; and conversely, what, if any, ingredients were nonessential. Similarly, it was desirable to determine whether Endo's medium would be equally applicable to another combination of slime mold and bacteria, e. g., *D. discoideum* in association with *Pseudomonas fluorescens*.

To accomplish these ends a series of media was prepared according to an Endo's formula, except that in each a single and different constituent, barring agar and distilled water, was omitted. Thus one medium was a complete Endo's medium except for the presence of peptone, a second complete except for the presence of lactose, etc. Since the Endo's medium first employed was a Difco[7] preparation, the formula used by that organization was employed as a basis for compounding the series of media (*7*).

In addition to the above, three other media were prepared and studied, namely, a complete Endo's medium; a medium of the same nutrient composition (1.0 percent each of peptone and lactose) but without sulphite and with eosin and methylene blue substituted for basic fuchsin;[8] and a medium which contained 1.2 percent of lactose and 0.4 percent of peptone but neither inorganic salts nor dyes.

Duplicate Petri plates of the several media were inoculated with *Escherichia coli*, five colonies being established per plate. At the same time, duplicate plates of the complete Endo's medium, the eosin-methylene blue medium, and the lactose-peptone medium were similarly inoculated with *Pseudomonas fluorescens*. Pure spores of *Dictyostelium discoideum*, obtained in the manner described in an earlier paper (*19*), were introduced into the resulting colonies of bacteria at the end of 2 days. Incubation throughout the experiment was at 20° to 23° C.

Within 2½ days after the introduction of pure spores of *Dictyostelium discoideum*, the central areas of all bacterial colonies had been cleared by the feeding and growing myxamoebae; and in all cultures with *Escherichia coli*, except in colonies on the Endo's medium minus lactose, pseudoplasmodia were present and young sorocarps were developing. In colonies of *Pseudomonas fluorescens* there was evidence of growth and multiplication of myxamoebae, but the cultures were plainly less vigorous than those with *E. coli* on identical media.

The growth and development of *Dictyostelium discoideum* in rich and favorable bacterial colonies such as those of *Escherichia coli* upon media containing both peptone and lactose (fig. 1) was in all cases much more luxuriant than reported previously for hay-infusion agar (*19*), and, in general, proceeded in the following manner. Within 20 to 24 hours after the spores were added, the first macroscopic sign of growth of the myxamoebae became evident as small centrally placed areas in which the bacteria had been noticeably consumed. During the following day these areas were gradually extended and when viewed under a low-power binocular microscope appeared finely and distinctly punctate owing to the refraction of light by the countless myxamoebae present. When the bacteria had been completely consumed in the colony centers, the myxamoebae commenced to form pseudoplasmodia (fig. 1, *A*, *b*). These did not form by the inflowing of long, extended, and ramifying streams of myxamoebae, as had previously been described as typical of this and related slime molds, but rather by a blocking off of the myxamoebae into irregular masses that subsequently evolved into elongate, cylindrical pseudoplasmodia that in form and size were characteristic of *D. discoideum* (fig. 1, *A*, *a*). At the same time, adjacent to and surrounding these centrally cleared areas were zones in which the myxamoebae were actively

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[6] It is, of course, recognized that peptones vary both in composition and in nutritive value, but this difficulty can be reduced to a minimum by using a product of high purity, and by using the same brand of peptone for all experiments where any comparison of results is anticipated. In this and in all succeeding experiments, the writer employed a peptone prepared and marketed by the Digestive Ferments Co. under the trade name "Bacto-Peptone"

[7] Product of the Digestive Ferments Co.

[8] A medium prepared according to a formula published by Levine (*11*).

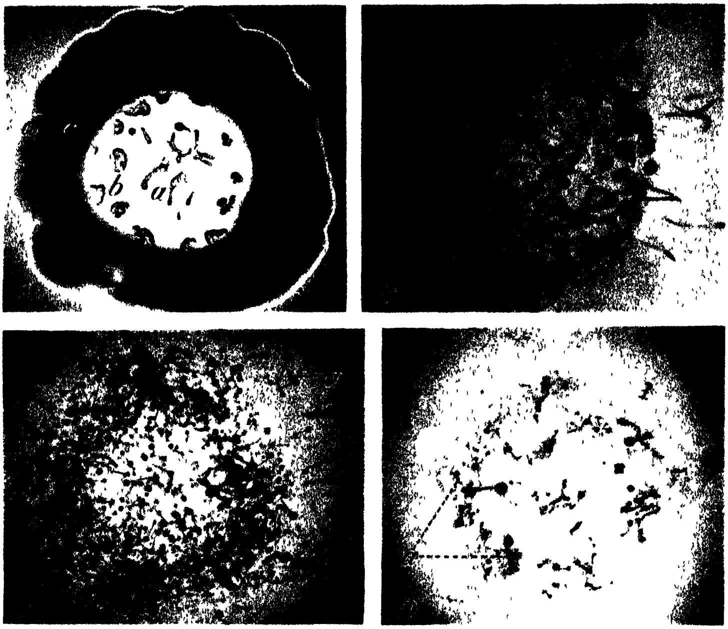

FIGURE 1.—Growth of *Dictyostelium discoideum* in association with *Escherichia coli* and *Pseudomonas fluorescens* upon media containing lactose and peptone. *A*, 2-day-old culture of *Dictyostelium* with *E. coli* upon eosin-methylene blue agar; *a*, Pseudoplasmodia ready to begin migration; *b*, forming pseudoplasmodia; *c*, zone in which myxamoebae are actively feeding; *d*, zone into which myxamoebae have not yet advanced. × 3. *B*, 3½-day-old culture with *E. coli* upon lactose (1.2 percent)-peptone (0.4 percent) medium; bacterial colony almost wholly consumed by myxamoebae which for the most part have now formed pseudoplasmodia or sorocarps. × 3. *C*, 8-day-old culture with *E. coli* upon Endo's medium minus fuchsin; bacterial colony wholly consumed by myxamoebae which subsequently formed (*a*) sorocarps of normal pattern. × 3. *D*, 8-day-old culture with *Pseudomonas fluorescens* upon eosin-methylene blue agar; bacterial colony consumed but produced (*a*) sorocarps of abnormal pattern, indicating presence of conditions not entirely favorable to the slime mold. × 3. (Photographs by Frank White.)

feeding and vegetating (fig. 1, *A*, *c*). In such colonies, therefore, the vegetative stage (i. e., the growth and multiplication of individual and separate myxamoebae) and the fruiting stage (i. e., the organization of myxamoebae into pseudoplasmodia and the subsequent formation of sorocarps) proceeded simultaneously and almost side by side; and as the former phase moved toward the colony edge the latter followed in its wake approximately 24 hours later.

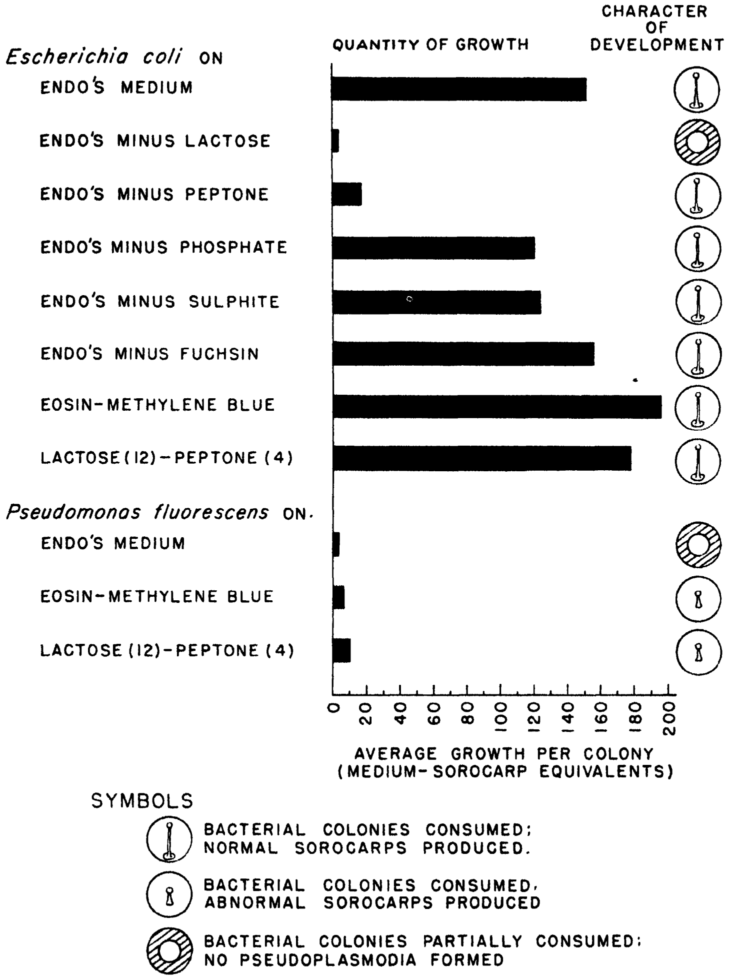

FIGURE 2.— Comparison of the amount of growth and the character of development of *Dictyostelium discoideum* in association with *Escherichia coli* and *Pseudomonas fluorescens* on Endo's and allied media.

A comparison of the total growth and of the character of development of the slime mold upon the different media and in association with the two bacterial hosts is presented in figure 2. For this comparison the average total number of large, medium, and small sorocarps per bacterial colony is expressed in the average number of medium-sorocarp equivalents, the necessary conversions being made by the method employed in earlier work (*19*). In this study it was found that the proportion of stalk or sorophore, to spore mass, or sorus, remained essentially the same regardless of the size of the sorocarp; therefore, by computing the volumes of their sori, sorocarps of different size could be readily represented in terms of a common unit such as a medium-sized sorocarp, for which unit the term "medium-sorocarp equivalent" was introduced.

Some striking and suggestive results were obtained from this experiment. *Dictyostelium discoideum* grew luxuriantly and developed normally in colonies of *Escherichia coli* only upon those media that contained both lactose and peptone. In the absence of peptone the growth of the bacterial associate was slight; and although the bacteria were completely consumed by the myxamoebae, the growth of *Dictyostelium* was correspondingly meager (fig. 2). On the other hand, *E. coli* grew excellently in the absence of lactose, but the myxamoebae grew very poorly and for a short time only, indicating that something inimical to the growth of the slime mold was being produced in these cultures (fig. 2). Phosphate and sulphite, additional ingredients of the complete Endo's medium, were found to be unnecessary, and through the omission of fuchsin a medium was secured that was equal to Endo's medium in every particular, and in the absence of color was markedly superior. That lactose and peptone were the essential ingredients of a favorable medium was clearly demonstrated by the excellent growth of *E. coli* and by the subsequent growth and normal development of *D. discoideum* which occurred upon the medium containing these components alone (fig. 2).

Equally indicative results were obtained from the cultures with *Pseudomonas fluorescens*. This bacterium flourished upon Endo's medium, but the slime mold grew poorly and but for a limited period, and only the central areas of the bacterial colonies were cleared by the myxamoebae. Thus these cultures were strikingly similar to those previously noted with *Escherichia coli* upon the lactose-free medium (fig. 2). Further, the cultures with *Ps. fluorescens* on the eosin-methylene blue medium and on the lactose-peptone medium presented evidence of unfavorable conditions approaching the above (fig. 2).

When it was recalled that *E. coli* could ferment lactose and *Ps. fluorescens* could not, the results of this experiment immediately suggested the following hypothesis: For the successful cultivation of *Dictyostelium discoideum* upon a peptone-rich medium, the peptone must be accompanied by a sugar that the associated bacteria can ferment. Furthermore, a balanced fermentation of the two nutritive ingredients of the medium must take place; that is, the carbon source, yielding acid products, and the nitrogen source, yielding alkaline products, must be broken down simultaneously, and the products of this dual fermentation must counteract one another.

STUDIES WITH PEPTONE-SUGAR MEDIA

Preliminary experiments designed to test the above-mentioned hypothesis supported its correctness, for it was uniformly found that upon media containing peptone and some sugar, good growth and normal development of *Dictyostelium discoideum* were limited to cultures in which the bacterial associate could ferment the carbohydrate contained in the medium. However, continued study along this line was considered worth while in order to understand adequately the combined influence of the composition of the culture medium and of the fermentations of the associated bacteria in their relation to the growth and development of *Dictyostelium*.

Media of the following composition were prepared with distilled water and solidified with 1.5 percent of agar: (1) A series of four media containing 1, 2, 4, and 12 g of peptone per liter as the sole nutrient; (2) a series of four media containing 12 g of dextrose and 1, 2, 4, and 12 g of peptone per liter as nutrients; (3) a series of four media containing 12 g of lactose and 1, 2, 4, and 12 g of peptone per liter as nutrients; and (4) a series of four media containing 12 g of sucrose and 1, 2, 4, and 12 g of peptone per liter as nutrients

The following species of bacteria were selected for this investigation upon the basis of their sugar fermentations: *Pseudomonas fluorescens*, No. 112,[9] which ferments dextrose but not lactose or sucrose; *Escherichia coli*, No. 107, which ferments dextrose and lactose but not sucrose; and *Escherichia communior* (Jackson) Bergey et al , No. 102, which ferments dextrose, lactose, and sucrose.

Duplicate Petri plates of the various media were inoculated with the selected bacteria, five colonies being planted in each plate. At the end of 3 days pure spores of *Dictyostelium discoideum* were introduced into four of the five colonies in each plate, the uninoculated fifth being kept as a measure of the bacterial growth. The cultures were incubated at 18° to 22° C. throughout the experiment.

A record of the growth of the three bacteria upon the several media was made at the time the spores of *D. discoideum* were introduced; and a tabulation of the quantity of growth and the character of development of *Dictyostelium* in colonies of the different bacteria upon the various media was made on the eighth or ninth day after the introduction of the spores. These records are summarized and presented in table 1.

A brief explanation of the terms used in table 1 is needed. The growth of bacteria upon the various media is listed first for each culture and is recorded in terms designated by the symbol *C* for a standard growth of *Escherichia coli* upon a medium containing 12 g of lactose and 4 g of peptone per liter, greater or less bacterial growth being denoted by a plus or minus sign, respectively. In these columns "growth" refers to the relative abundance of myxamoebae present, as evidenced by the extent to which the bacterial colonies were cleared or by the number of sorocarps produced. Colonies referred to are in all cases bacterial colonies, and the extent to which they were consumed by the myxamoebae is denoted by the degree of gross clearance. The character of sorocarps is given as normal and abnormal (fig. 3).

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[9] The carbohydrate fermentations of these particular cultures, together with several others used later in this investigation, were known from the unpublished work of N. R. Smith, senior bacteriologist in the Bureau of Plant Industry, from whom the cultures were obtained.

The growth of *Dictyostelium discoideum* in colonies of the three associated bacteria will be considered separately: (1) *Dictyostelium discoideum* grew moderately well in colonies of *Pseudomonas fluorescens* on media containing dextrose but not on media containing peptone alone or peptone together with lactose or sucrose. This fact becomes particularly interesting when it is recalled that dextrose is the only one of the three sugars employed that could be fermented by *Ps. fluorescens.* (2) Moderate to very good growth and normal development of *D. discoideum* occurred in colonies of *Escherichia coli* on media containing peptone and dextrose and peptone and lactose, but not on peptone alone or peptone and sucrose. Thus with *E. coli*, as with *Ps. fluorescens*, good growth and normal development of *Dictyostelium* occurred only on media containing sugars that could

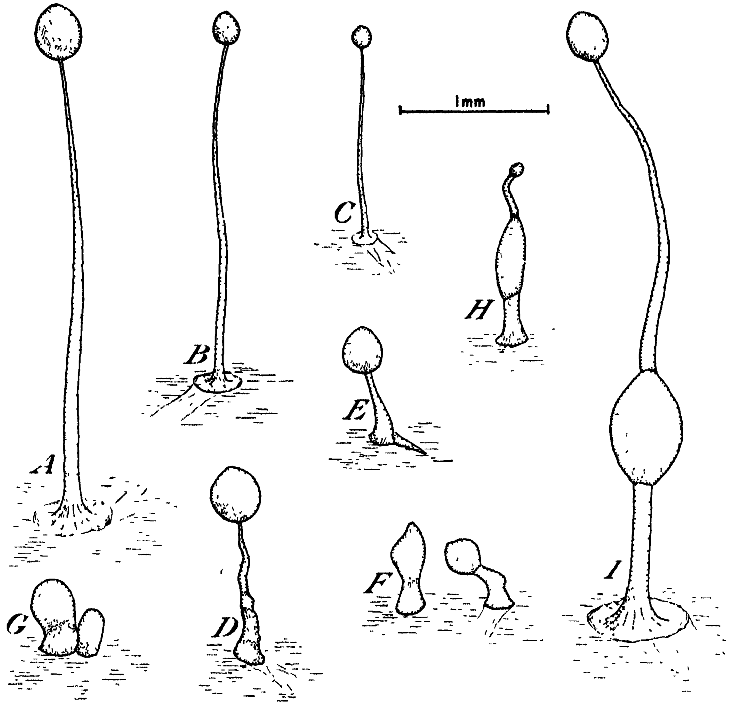


FIGURE 3.—Fruiting structures, or sorocarps, of *Dictyostelium discoideum*. *A–C*, Normal sorocarps of large (*A*), medium (*B*), and small (*C*) size; the ellipsoid head, or sorus, the upright, evenly tapering stalk, or sorophore, and the discoid base are clearly evident. *D–I*, Abnormal sorocarps: *D* and *E*, Sorocarps with relatively large sori, short, strongly tapering sorophores, and poorly formed basal disks; *F*, sorocarps with poorly differentiated sori and sorophores; *G*, masses of myxamoebae in which sori and sorophores have not been differentiated because of extremely unfavorable culture conditions; *H*, sorocarp of very abnormal form in which spores have not been produced; *I*, sorocarp in which the greater portion of the spore-forming myxamoebae produced a sorus a little below midway of the completed sorophore.

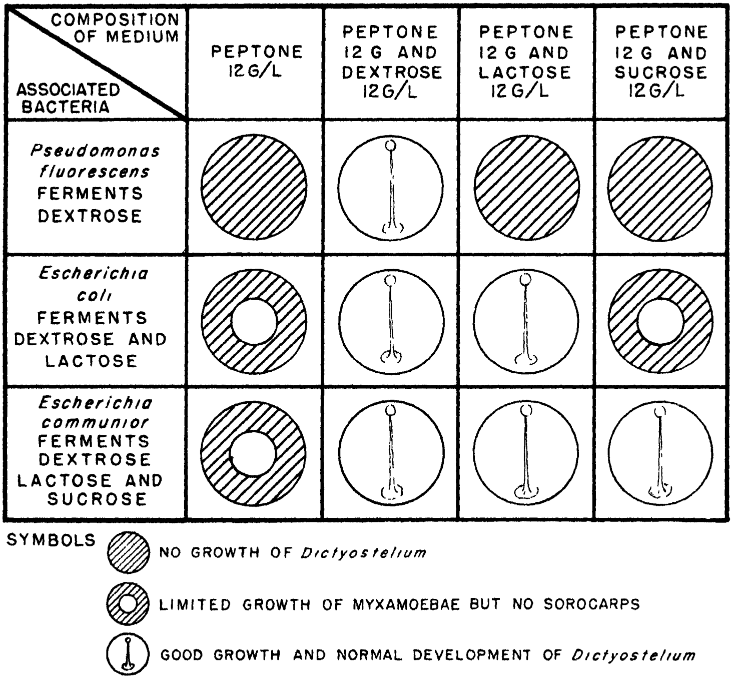
be utilized by the bacteria. (3) Moderate to very good growth and normal development of *D. discoideum* took place in colonies of *Escherichia communior* on all media except that containing peptone alone. Here again good growth and normal development of *D. discoideum* could be directly correlated with the specific sugar fermentations of the bacterial culture, for *E. communior* is able to utilize dextrose, lactose, and sucrose.

TABLE 1.—*Combined influence of the bacterial associate and of the medium upon the growth and development of Dictyostelium discoideum*

Designation and composition of medium (grams per liter)	Growth[1] of *Dictyostelium discoideum* with indicated bacterial associate		
	Pseudomonas fluorescens No. 112	*Escherichia coli* No 107	*Escherichia communior* No. 102
P1 (peptone 1, water 1,000, agar 15)	*C*– Growth poor; colonies cleared, sorocarps slightly abnormal.	*C*–. Growth fair; colonies cleared; sorocarps near normal.	
P2 (peptone 2 : 1,000).	*C*–. Growth poor, colonies cleared, sorocarps abnormal.	*C*. Growth fair; colonies cleared; sorocarps few, abnormal.	
P4 (peptone 4 : 1,000).	*C*– Growth poor; colonies partially cleared, few pseudoplasmodia, no sorocarps.	*C*. Growth fair; colonies cleared; sorocarps few, abnormal.	*C*. Growth poor, colonies cleared, sorocarps few, abnormal.
P12 (peptone 12 1,000).	*C*. No growth; spores ungerminated.	*C*+ Growth fair, colonies partially cleared, few pseudoplasmodia, no sorocarps.	*C*+. Growth very poor, colonies partially cleared; no pseudoplasmodia, no sorocarps.
D1 (dextrose 12, peptone 1 . 1,000)	*C*–. Growth fair; colonies largely cleared; sorocarps normal.	*C*–. Growth fair, colonies cleared; sorocarps normal.	
D2 (dextrose 12, peptone 2 . 1,000).	*C*–. Growth fair, colonies largely cleared; sorocarps normal.	*C*–. Growth fair; colonies cleared; sorocarps normal.	
D4 (dextrose 12, peptone 4 : 1,000)	*C*. Growth fair; colonies largely cleared; sorocarps normal.	*C*. Growth good; colonies cleared; sorocarps normal.	*C*. Growth good; colonies cleared; sorocarps normal
D12 (dextrose 12, peptone 12 : 1,000)	*C*+. Growth good, colonies partially cleared; sorocarps normal.	*C*+. Growth very good; colonies cleared; sorocarps normal.	*C*+. Growth very good, colonies cleared; sorocarps normal.
L1 (lactose 12, peptone 1 : 1,000).	*C*––. Growth fair; colonies cleared; sorocarps normal.	*C*–. Growth fair; colonies cleared; sorocarps normal.	
L2 (lactose 12, peptone 2 : 1,000).	*C*–. Growth fair; colonies cleared; sorocarps normal and abnormal.	*C*. Growth fair; colonies cleared; sorocarps normal.	
L4 (lactose 12, peptone 4 : 1,000).	*C*–. Growth poor; colonies largely cleared; sorocarps abnormal.	*C*. Growth good; colonies cleared; sorocarps normal.	*C*. Growth good; colonies cleared, sorocarps normal.
L12 (lactose 12, peptone 12 : 1,000).	*C*. No growth; spores ungerminated.	*C*+. Growth very good, colonies cleared; sorocarps normal.	*C*+. Growth very good, colonies cleared; sorocarps normal.
S1 (sucrose 12, peptone 1 : 1,000).	*C*––. Growth fair, colonies cleared; sorocarps normal.	*C*––. Growth fair; colonies cleared; sorocarps normal	*C*–. Growth poor; colonies cleared; sorocarps normal.
S2 (sucrose 12, peptone 2 : 1,000).	*C*–. Growth fair; colonies cleared; sorocarps few, abnormal.	*C*–. Growth fair; colonies cleared; sorocarps abnormal.	*C*–. Growth fair, colonies cleared; sorocarps mostly normal.
S4 (sucrose 12, peptone 4 : 1,000).	*C*–. Growth fair; colonies cleared; pseudoplasmodia; very few sorocarps, abnormal.	*C*–. Growth fair; colonies largely cleared; sorocarps few, abnormal.	*C*. Growth good; colonies cleared; sorocarps mostly normal.
S12 (sucrose 12, peptone 12 : 1,000).	*C*. No growth Spores ungerminated.	*C*. Growth fair; colonies partially cleared; no sorocarps.	*C*+. Growth very good, colonies cleared; sorocarps normal.

[1] See text for fuller explanation of terms used.

Turning now from the growth of *Dictyostelium discoideum* with specific bacteria to more general considerations, some additional points are worthy of attention. (1) Although the presence of an available sugar in the medium did not lead to an appreciable increase in the amount of bacterial growth (table 1), its decomposition by the bacteria obviously supplied something which rendered the bacterial colonies favorable to the slime mold, for in no case did good growth and normal development of *D. discoideum* occur where the peptone was not accompanied by a sugar that could be broken down by the associated bacteria (fig. 4). (2) In cultures where the sugar could be utilized by the associated bacteria, the amount of growth of *Dictyostelium* increased progressively with the concentration of peptone, and, therefore, as the quantity of bacterial growth increased. Conversely, in cultures where there was no sugar, or an unavailable



COMPOSITION OF MEDIUM / ASSOCIATED BACTERIA	PEPTONE 12G/L	PEPTONE 12 G AND DEXTROSE 12G/L	PEPTONE 12 G AND LACTOSE 12G/L	PEPTONE 12 G AND SUCROSE 12G/L
Pseudomonas fluorescens FERMENTS DEXTROSE	▨	Ⓘ	▨	▨
Escherichia coli FERMENTS DEXTROSE AND LACTOSE	◍	Ⓘ	Ⓘ	◍
Escherichia communior FERMENTS DEXTROSE LACTOSE AND SUCROSE	◍	Ⓘ	Ⓘ	Ⓘ

SYMBOLS
▨ NO GROWTH OF *Dictyostelium*
◍ LIMITED GROWTH OF MYXAMOEBAE BUT NO SOROCARPS
Ⓘ GOOD GROWTH AND NORMAL DEVELOPMENT OF *Dictyostelium*

FIGURE 4.—Diagram illustrating the combined influence of the medium and of the bacterial associate upon the growth and development of *Dictyostelium discoideum.*

sugar, the growth of the slime mold decreased progressively with an increase in peptone, and therefore with an increase in bacterial growth (table 1). (3) Good growth and normal development of *D. discoideum* did not occur in colonies of any of the three bacteria upon media containing only peptone or peptone and an unavailable sugar. However, more nearly normal development occurred upon low than upon higher concentrations of peptone (table 1). This clearly indicated that with increasing amounts of peptone, and in the absence of fermentable sugars, the associated bacteria produced some substance inimical to the growth and normal development of *Dictyostelium* in progressively greater quantity. (4) In cultures upon media containing peptone and an available sugar, the growth of *Dictyostelium discoideum* was proportional to the quantity of bacteria present and was limited solely by the abundance of the food supply; whereas, in cultures upon media containing peptone alone or in conjunction with an unfermentable sugar, factors other than the abundance of the food supply governed the growth of the slime mold, and in these latter cultures it was invariably observed that the character of development (i. e., the presence or absence of sorocarps, or the form of sorocarps produced) afforded a reliable measure of the favorableness or unfavorableness of culture conditions to *D. discoideum* (p. 189–190).

After the completion of the work just described, a supplementary experiment wholly substantiated the results already obtained regarding the influence of the bacterial associate and of the medium upon the growth and development of *Dictyostelium discoideum*. Duplicate plates of a medium containing 12 g of peptone and 12 g of soluble starch per liter were inoculated with *Pseudomonas fluorescens*, *Escherichia coli*, *E. communior*, and *Cellulomonas liquata* (Kellerman et al.) Bergey et al. Of these species only the latter is able to hydrolyze starch. Pure spores of *D. discoideum* were introduced into the bacterial colonies when 2 days old. *Dictyostelium* failed to grow in colonies of *Ps. fluorescens;* grew poorly but failed to form pseudoplasmodia and sorocarps in colonies of *E. coli* and *E. communior;* but grew well and produced normal, well-formed sorocarps (fig. 3, *A*, *C*) in colonies of *C. liquata*. Thus, another case was presented where the presence of good growth and normal development could be traced to the ability of the associated bacteria to ferment the carbohydrate contained in the medium.

For the successful cultivation of *Dictyostelium discoideum* upon peptone-sugar media the importance of a balanced fermentation of the component nutrients by the associated bacteria cannot be overemphasized. In the experiments just considered, the writer employed media containing up to 1.2 percent each of peptone and some carbohydrate fermentable by the accompanying bacteria. However, this concentration of nutrients was chosen arbitrarily, and supplementary experiments have shown that media containing 1 percent each of peptone and a fermentable carbohydrate yield equally satisfactory results. Furthermore, the concentrations may be augmented if the nutrients are increased proportionately, e. g., luxuriant growth and normal development of *D. discoideum* occur in association with *Escherichia coli* upon media containing 2.5 percent each of peptone and lactose, whereas poor growth and abnormal development occur where the concentration of peptone is increased above 1.2 percent without a corresponding increase in lactose.

Finally, a point deserving particular attention was brought out in certain of the writer's cultures through their occasional contamination with fungi belonging to the genera *Penicillium* and *Aspergillus*. It has been repeatedly noted that the growth of *Dictyostelium discoideum* was limited or wholly inhibited in colonies of bacteria grown upon media containing peptone and some sugar which was unavailable to the associated bacteria (fig. 4). However, when fungus contaminants occurred in such cultures, *D. discoideum* grew well and developed normally in colonies of bacteria adjacent to the fungus colonies. Since it was known that these fungi were able to break down the sugars contained in the media, the following explanation was suggested: In breaking down the sugars the fungi produced something which counteracted the toxic products formed from the peptone by the bacteria; for in these cultures the formation of such toxic products could not have been inhibited by the fungi, since they came in at the time of inoculation with *Dictyostelium* and after the bacteria had virtually ceased growing. Further, since it was known that the bacteria in question in breaking down peptone produced ammonia and that this in turn rendered the medium alkaline, and since, on the other hand, it was known that the fungi in breaking down sugars produced acids which neutralized the ammonia and thus tended to keep the medium from becoming alkaline, it became apparent that the toxic condition existing in the bacterial colonies might well consist of an alkaline reaction resulting from the production and accumulation of ammonia.

Additional observations lent supporting evidence to this hypothesis, namely: (1) Colonies of bacteria grown upon rich peptone media are toxic to *Dictyostelium discoideum*, whereas the bacterial cells which comprise such colonies are nontoxic, thus indicating that the noxious principle, like ammonia, is a diffusible substance liberated from the peptone as the result of bacterial growth; (2) colonies of bacteria become increasingly toxic as the concentration of peptone is augmented in the absence of a fermentable carbohydrate, whereas they remain uniformly nontoxic as the percentage of peptone is increased in the presence of such a carbohydrate (table 1). Preliminary tests supported the hypothesis, for it was found that the reaction of bacterial colonies in which good growth and normal development of *D. discoideum* occurred was slightly acid (pH 6.5±), whereas the reaction of colonies supporting very limited growth of the slime mold was alkaline (pH 8.0±).

INFLUENCE OF HYDROGEN-ION CONCENTRATION

The results of the foregoing studies suggested that bacterial colonies of an alkaline reaction were toxic to *Dictyostelium discoideum*, whereas colonies of a neutral or acid reaction favored its growth and development; or, stated in another manner, they indicated that good growth and normal development of the slime mold were dependent upon the existence of a favorable hydrogen-ion concentration in the colonies of host bacteria. Since such a hypothesis lent itself readily to investigation, a series of experiments was subsequently performed for the specific purpose of determining the relationship between hydrogen-ion concentration and the culture conditions favoring optimum growth and development of *D. discoideum*.

METHODS

In these experiments the determination of the hydrogen-ion concentration of bacterial colonies on the culture plate constituted a most important phase of the investigations; and since the same procedure was followed in all experiments, a brief review of the simple colorimetric method employed for these determinations will be given forthwith. A preliminary test was first made with bromothymol blue to determine whether the colony and the adjacent medium were acid or alkaline in reaction, and once this fact was established, drops of other indicators with ranges covering the suspected pH were added. Two or more indicators were used in making each determination, and by comparing the pH values signified by the different indicators an approximation of the true pH value was obtained. It is recognized that this method could not give exact pH readings, but the values obtained and recorded in the following experiments are believed to be correct to within approximately 0.2± of a pH unit, which was considered sufficiently accurate for the purpose of these experiments.

It should be remembered that in cultures of bacteria, or bacteria and *Dictyostelium*, the pH varies appreciably in different zones within the culture plate. It is evident that if bacteria are breaking down peptone and liberating ammonia the reaction within the colonies will be more alkaline than at some distance away. And, conversely, if the sugar is broken down rapidly the reaction within the colonies will be more acid than in areas removed from them. However, satisfactory determinations could not be made regularly within the bacterial colonies because of their opacity, uneven contours, and, in some cases, color. Nor could they be made satisfactorily in colonies of bacteria and *Dictyostelium* because of the irregularity with which the drops of indicator spread owing to the presence of the fruiting structures of the slime mold. Therefore, in all cases, except where otherwise specifically noted, the pH values recorded in the following experiments are for zones of approximately 1 cm in width adjacent to the colonies of bacteria, or bacteria and *Dictyostelium*, as the case may be.

ADDITION OF FUNGI

In an earlier experiment it was noted that upon peptone-sugar media *Dictyostelium discoideum* grew and developed normally in cultures contaminated by fungi, whereas it failed to grow in uncontaminated but otherwise similar cultures. A likely explanation of this phenomenon was believed to reside in a difference in the hydrogen-ion concentration of bacterial colonies in the presence and in the absence of fungi. Accordingly, an experiment was undertaken to determine the difference in hydrogen-ion concentration between favorable cultures in the presence of fungi and of unfavorable ones in their absence, and to investigate generally the relationship between the members of this triple association of *Dictyostelium*, bacteria, and fungi.

For this investigation agar media containing 1.2 percent each of peptone and lactose, peptone and sucrose, and peptone and starch were prepared, the initial reaction of each medium being pH 6.8 to 7.0. In order to follow the pH changes resulting from the growth of bacteria and fungi, a nontoxic indicator, bromocresol purple,[10] was incorporated in the media.

Two host bacteria were selected upon the basis of their carbohydrate fermentations, namely: *Pseudomonas fluorescens*, which ferments neither lactose nor sucrose and does not hydrolyze starch; and *Escherichia coli*, which ferments lactose but not sucrose and does not hydrolyze starch. *Penicillium chrysogenum* Thom was selected as a fungus associate because of its known vigor and its ability to utilize each of the carbohydrates contained in the media.

Plates of the different media were inoculated with bacteria as shown in figure 5. Two days later the resulting colonies were seeded with pure spores of *Dictyostelium*, and immediately thereafter *Penicillium chrysogenum* was introduced into certain colonies by streaking spores along lines parallel to the rows of bacterial colonies, while no fungus was introduced into other cultures kept as controls.

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[10] Bromocresol purple has a pH range of 5.2 to 6.8 and a color change from yellow to purple. It was used in a concentration of 20 cc of a 0.04 percent aqueous solution per liter

A record of the growth of *Dictyostelium discoideum* in the different cultures was made on the tenth day after its introduction and is presented in table 2.

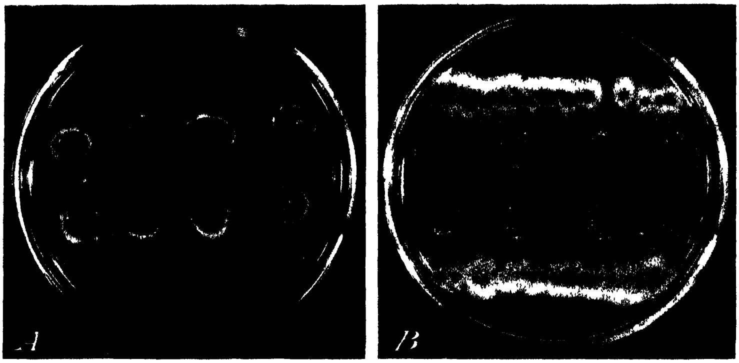

FIGURE 5.—Influence of an added fungus, *Penicillium chrysogenum*, upon the growth of *Dictyostelium discoideum* in association with *Escherichia coli* on peptone-sucrose media: *A*, No growth of *Dictyostelium* in the absence of the fungus; *B*, good growth of the slime mold in the presence of the fungus. Age: Bacterial colonies 7 days, *Dictyostelium* and *Penicillium* 5 days. × ½. (Photographs by Frank White.)

TABLE 2.—*Growth of Dictyostelium discoideum in the presence and in the absence of an added fungus, Penicillium chrysogenum*

Bacterial associate	Fungus added	Composition of medium (grams per liter)	Growth of *Dictyostelium discoideum*		
			Growth of myxamoebae	Clearance of bacterial colony	Sorocarp formation
Pseudomonas fluorescens.	*Penicillium chrysogenum*	Peptone, 12, lactose, 12.	Good	Largely cleared	Abundant sorocarps, mostly normal in size and pattern
	do	Peptone, 12, sucrose, 12	do	do	Abundant sorocarps, mostly normal
	None	Peptone, 12, lactose, 12.	None	None	None
	do	Peptone, 12, sucrose, 12	do	do	Do
Escherichia coli.	*Penicillium chrysogenum.*	do	Very good	Cleared	Very abundant, normal
	do	Peptone, 12, starch, 12	Good	Largely cleared	Abundant, mostly normal.
	None	Peptone, 12, sucrose, 12.	None	None	None
	do	Peptone, 12, starch, 12	Slight	Slightly cleared.	Very few sorocarps, very abnormal

The results of the experiment can be summarized as follows: *Dictyostelium* grew well in colonies of *Pseudomonas fluorescens* on lactose-peptone and sucrose-peptone media in the presence of *Penicil-*

lium chrysogenum, but failed to grow in colonies of this bacterium on the same media in the absence of the fungus. Similarly, good growth and normal fructification of *Dictyostelium* occurred in colonies of *Escherichia coli* on sucrose-peptone (fig. 5, *B*) and starch-peptone media in the presence of *Penicillium*, but in its absence *Dictyostelium* grew poorly or not at all (fig. 5, *A*). Thus, in each case, good growth and normal development of the slime mold could be attributed to the favorable influence of the added fungus. The following observations indicate that this influence was exerted through the creation of an acid reaction in the medium and in the colonies of host bacteria. The media were originally purple in color owing to their neutral reaction and the presence of the indicator, and they remained so, becoming more alkaline as the bacteria grew, until the added fungus began growing. As this occurred, however, acid was produced and a zone of yellow appeared, developing first directly along the line of fungus growth but subsequently spreading outward in advance of the hyphal tips. As the acid zone approached the bacterial colonies, *Dictyostelium* began to grow, and by the time the zone reached the colonies most of the bacteria had been consumed, pseudoplasmodia had formed, and normal sorocarps were developing. In cultures to which no fungus was added, the media remained purple throughout the experiment, indicating that the reaction either remained neutral or became alkaline. Subsequent tests in cultures on sucrose-peptone media without bromocresol purple showed that in the absence of fungi the reaction in colonies of *Escherichia coli* reached pH 8.0 to 8.2, and in those of *Pseudomonas fluorescens* it reached pH 8.4 to 8.6; and in neither case did the slime mold gr [illegible] ies of host bacteria, the absence of good gro [illegible] pment of *Dictyostelium discoideum* could be [illegible] aline reaction, whereas their presence could b [illegible] d reaction.

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The present study [illegible] teresting point. Since *Dictyostelium discoide* [illegible] not at all in the absence of bacteria (*19*), ~~when studying this slime mold~~ or related forms one must of necessity deal with an association of two organisms; and in such a dual association the bacteria serve as hosts for the *Dictyostelium* (*19*). In the present case, however, three organisms were involved; and although the bacteria still acted as hosts for the slime mold they did so only in the presence of a third organism, a fungus, which entered the association indirectly by altering the reaction of the bacterial colonies through changing the culture medium. The bacteria and the fungus obviously acted upon the media in separate and different capacities, and the subsequent growth and normal development of *Dictyostelium* depended upon the acid products formed from the carbohydrate by the fungus counteracting the alkaline products liberated from the peptone by the bacteria. When only the peptone was broken down, ammonia accumulated, the bacterial colonies became quite alkaline in reaction, and the growth of *Dictyostelium* was inhibited; whereas, when the carbohydrate was subsequently broken down, the reaction of the bacterial colonies shifted from alkaline to acid, and in this new environment the slime mold was able to feed, grow, and form normal sorocarps. Thus was demonstrated a striking example of "micro-ecology," or the interrelationships of micro-organisms in culture.

ADDITION OF STERILE ACID

Earlier studies indicated that good growth and normal development of *Dictyostelium discoideum* were dependent upon the existence of an acid reaction in the colonies of associated bacteria and that the toxicity of unfavorable colonies consisted in their alkaline reaction. In the preceding experiment such an acid reaction was established by the fermentation of carbohydrates by a fungus which was introduced into the cultures for that specific purpose. In the experiments next to be considered, it was obtained by the addition of free acid to cultures in which bacteria had already grown. Thus the influence of hydrogen-ion concentration apart from any carbohydrate fermentation could be determined.

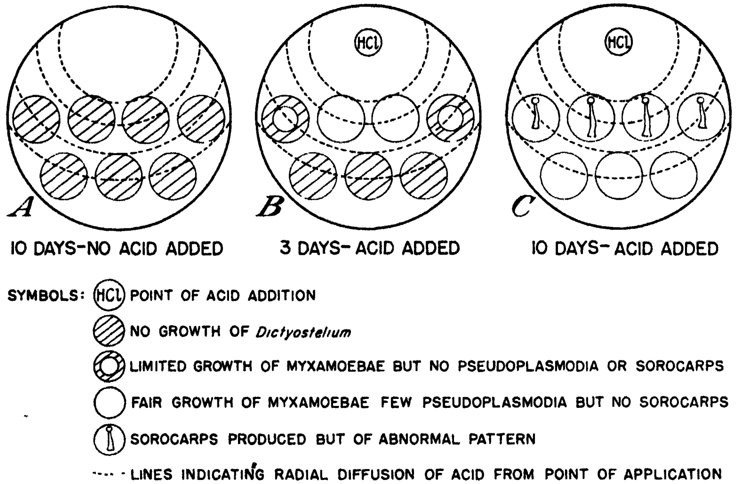

HCl
HCl
A
B
C
10 DAYS-NO ACID ADDED
3 DAYS-ACID ADDED
10 DAYS-ACID ADDED
SYMBOLS: HCl POINT OF ACID ADDITION
NO GROWTH OF Dictyostelium
LIMITED GROWTH OF MYXAMOEBAE BUT NO PSEUDOPLASMODIA OR SOROCARPS
FAIR GROWTH OF MYXAMOEBAE FEW PSEUDOPLASMODIA BUT NO SOROCARPS
SOROCARPS PRODUCED BUT OF ABNORMAL PATTERN
LINES INDICATING RADIAL DIFFUSION OF ACID FROM POINT OF APPLICATION

FIGURE 6.—The influence of added acid upon the growth of *Dictyostelium discoideum* in colonies of *Escherichia coli* on peptone-sucrose agar. *A*, No acid added; no growth of *Dictyostelium*, the spores remaining ungerminated 10 days after inoculation. *B*, 0.5 cc sterile N/2 HCl added during 3-day period. Bacterial colonies nearest the point of acid application have been consumed by the *Dictyostelium*, those somewhat more removed have been partially consumed, while those farthest removed remain intact; thus the growth of the slime mold can be correlated with the outward diffusion of the added acid. *C*, Same culture as *B*, but 7 days older. All bacterial colonies have been consumed by the *Dictyostelium*, but sorocarps have developed only in the four nearest the point of acid application.

Plates of an agar medium containing 1.2 percent each of peptone and sucrose were inoculated with *Escherichia coli* in the manner shown in figure 6, *A*; and 3 days later the resulting bacterial colonies were inoculated with pure spores of *Dictyostelium discoideum*. Immediately thereafter 2 drops of steam-sterilized N/2 hydrochloric acid were added to duplicate cultures, as shown in figure 6, *B*, and additional acid up to approximately 0.5 cc was added over a period of 3 days, 1 or 2 drops being added at 12-hour intervals. The results of this preliminary experiment are diagrammatically shown in figure 6. *Dictyostelium discoideum* failed to grow in cultures to which no acid was added; but it did grow in cultures to which acid was added, and greater growth and more nearly normal development took place in the bacterial colonies closest to the points of acid application.

Since *Dictyostelium* failed to grow in control cultures to which no acid was added, its growth in acidified cultures could be attributed solely to the favorable influence of the added acid. On the other hand, since *Dictyostelium* grew best in bacterial colonies nearest the sites of acid application but even there was abnormal in its development (fig. 6, *C*), it seemed probable that the quantity of acid used was insufficient to counteract completely the alkaline reaction of the bacterial colonies. Accordingly, in the succeeding experiment increased quantities of sterile acid were employed.

For this succeeding investigation, as for the preliminary experiment just considered, agar media containing 1.2 percent each of peptone and sucrose were employed; and, as in the earlier study with added fungi, the indicator bromocresol purple was included in the medium in order that the changes in the hydrogen-ion concentration of the cultures could be more easily followed.

Escherichia coli and *Pseudomonas fluorescens* were again selected as bacterial associates. Neither species can ferment sucrose, and from earlier studies it was known that upon peptone-sucrose media these bacteria produced colonies in which *Dictyostelium* could not ordinarily grow. Incubation of the cultures was at 20° to 23° C. throughout the experiment.

To understand better the influence of the acid, the quantitative relation between the medium and the added acid was carefully considered. In all cases Petri plates containing 20 cc of medium were used, and different amounts of acid were subsequently added. Eight plates were inoculated with *Escherichia coli* and a like number with *Pseudomonas fluorescens*. At the end of 3 days the resulting colonies of bacteria were inoculated with pure spores of *Dictyostelium discoideum*, and immediately thereafter the addition of acid was begun. Of each series of eight plates two were kept as controls and received no acid, two received 0.5 cc, two 1.0 cc, and two 1.5 cc of steam-sterilized N/2 hydrochloric acid. In each case the acid was added in five equal applications, the first being made at the time the spores were introduced and the three successive applications being made at 12-hour intervals thereafter. The fifth and final application was delayed in all cases until the fourth day after spore introduction, and was then added primarily to maintain the reaction then obtaining in the cultures rather than to render them more acid. In all cases the acid was added by means of sterile 1-cc pipettes graduated in 0.01-cc units.

A record of the growth of *Dictyostelium discoideum* in the different cultures was made on the eighth day after the introduction of the slime mold and is presented in table 3, while a graphic comparison of the amount of growth under the different culture conditions is shown in figure 7.

Dictyostelium discoideum failed to grow in colonies of *Escherichia coli* or *Pseudomonas fluorescens* in the absence of added acid; while, within the limits of the experiment, the growth of *Dictyostelium* increased progressively as the quantity of added acid was augmented (fig. 7, *A*, *B*). In this connection, however, it should be noted that in cultures to which 1.5 cc of sterile N/2 hydrochloric acid was added portions of the bacterial colonies nearest the sites of acid application were rendered very acid, and in these strongly acid zones *D. discoideum* failed to grow (fig. 8, *B*, *C*).

In another experiment paralleling that described above, similar quantities of acid were added to cultures grown upon media containing peptone alone, with results essentially duplicating those observed in cultures on peptone-sucrose media.

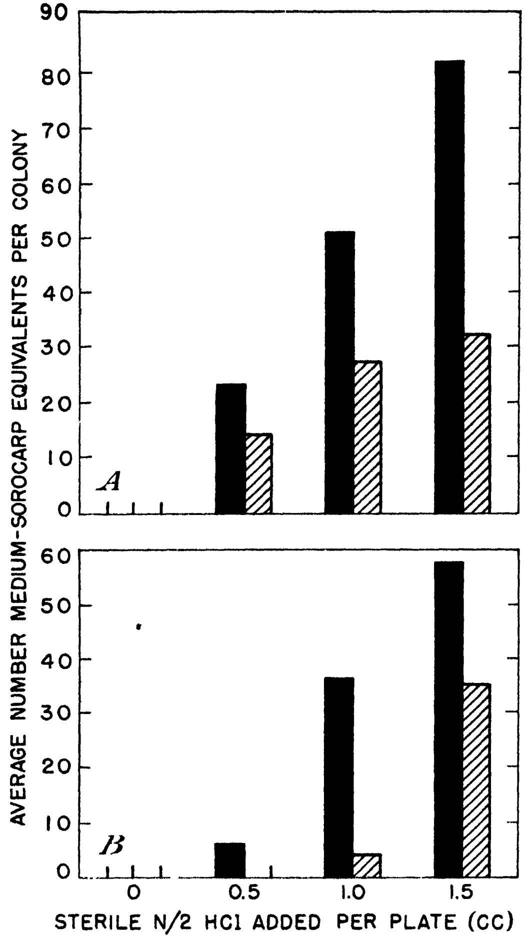


FIGURE 7. Comparative growth of *Dictyostelium discoideum* in association with *Escherichia coli* (*A*) and with *Pseudomonas fluorescens* (*B*) upon peptone-sucrose agar plates to which different quantities of sterile N/2 HCl were added. Solid columns represent colonies adjacent to added acid; broken columns, colonies farther removed from added acid.

Particular quantities of acid permitted a greater growth of *Dictyostelium* when added to cultures of *Escherichia coli* than when added to cultures of *Pseudomonas fluorescens* (fig. 7). This, however, was to be expected, since *Ps. fluorescens* is the more active proteolytic organism of the two and therefore produces more ammonia than *E. coli* on media containing peptone.

In general the pseudoplasmodia migrated toward the acid portions of the culture plates. This response is well illustrated in figure 8,

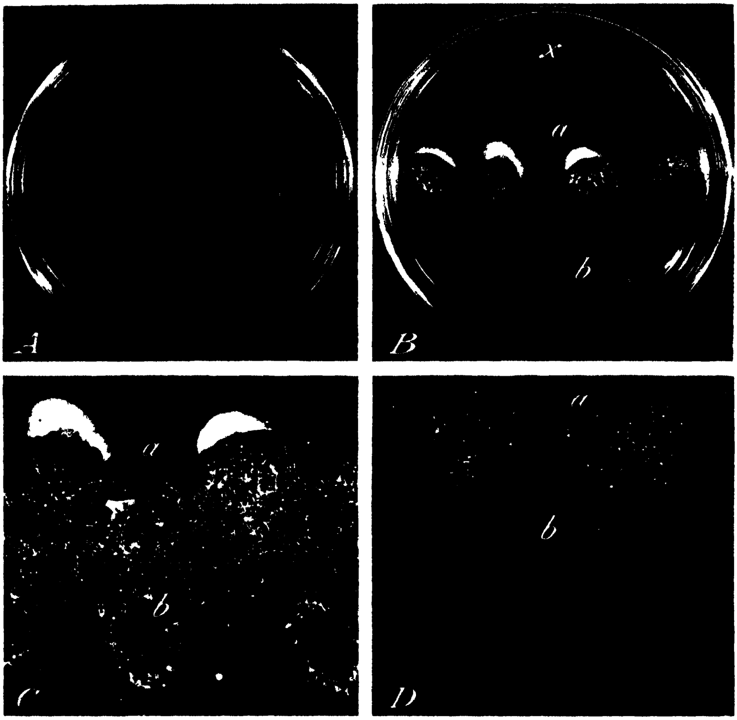


FIGURE 8.—Influence of added acid upon the growth and development of *Dictyostelium discoideum* in association with *Escherichia coli* on peptone-rich agar media containing the indicator bromocresol purple. *A*, Peptone-sucrose medium; no acid added; no growth of *Dictyostelium;* 10 days after inoculation; × ½ *B*, Peptone-sucrose medium; acidified culture, 1.5 cc of sterile N/2 HCl added at *x* during 3-day period following inoculation with *Dictyostelium;* four bacterial colonies in acid zone (*a*) largely consumed (crescent-shaped uncleared zones too acid for *Dictyostelium*) and abundant sorocarps present; colonies in the neutral or alkaline zone (*b*) partially consumed, many pseudoplasmodia present; 5 days; × ½. *C*, Central area of *B;* strongly acid portions (*a*) of bacterial colonies still untouched, remainder (*b*) completely consumed by the slime mold; 10 days; × 1⅕. *D*, Peptone medium; acidified culture, 1.0 cc of sterile N/2 HCl added as in *B*. concentration of sorocarps along the border line between the acid zone (*a*) and the neutral or alkaline zone (*b*) of the culture plate indicates a positive chemotactic response to acid by the migrating pseudoplasmodia; 10 days after inoculation; × 1⅕. (Photographs by Frank White.)

C and *D*, where the sorocarps are seen clustered at the junction of the acid and alkaline zones in the medium. Since the cultures were grown in continuous darkness, this can apparently be regarded as a true chemotactic response.

TABLE 3.—*Growth of Dictyostelium discoideum in colonies of Escherichia coli and Pseudomonas fluorescens on peptone-sucrose medium in the presence of different quantities of added sterile N/2 hydrochloric acid*

Bacterial associate	Quantity of N/2 HCl added per plate	Area of plates rendered acid	Growth of *Dictyostelium discoideum*											
			Average growth per colony in row adjacent to site of acid application						Average growth per colony in row removed from site of acid application					
			Extent of colony clearance	Sorocarps				Typical sorocarps	Extent of colony clearance	Sorocarps				Typical sorocarps
				Large	Medium	Small	Medium-sorocarp equivalents			Large	Medium	Small	Medium-sorocarp equivalents	
	Cc	*Percent*		*Number*	*Number*	*Number*	*Number*	*Percent*		*Number*	*Number*	*Number*	*Number*	*Percent*
Escherichia coli	0	0	None	0	0	0	0	0	None	0	0	0	0	0
	.5	0	Complete	3-4	8-10	8-10	23	20	Complete	2-3	4-6	3-4	14	0
	1.0	35	do	8-10	15-20	10-15	51	65	do	4-5	8-10	10-12	27	20
	1.5	65	Largely complete[1]	12-15	20-25	10-15	82	75	do	5-6	10-12	12-15	32	40
Pseudomonas fluorescens	0	0	None	0	0	0	0	0	None	0	0	0	0	0
	.5	0	Slight	0-1	3-4	4-5	6	0	do	0	0	0	0	0
	1.0	25	Partial	6-8	10-12	4-6	37	70	Slight	0	[2] 3-4	0	4	0
	1.5	75	do[1]	10-12	15-20	8-10	58	50	Complete	6-8	8-10	8-10	35	40

[1] In colonies nearest the sites of acid application crescent-shaped zones of bacterial colonies became very acid in reaction and were not consumed by the feeding myxamoebae (fig. 8, *B*, *C*).

[2] Irregular masses of myxamoebae; no true sorocarps formed

Greater growth of the slime mold occurred in the bacterial colonies adjacent to the points of acid application than in those more removed from these sites; and in all cases a greater percentage of the sorocarps developing near the boundary of the acid zone was of normal pattern than of those developing elsewhere (fig. 8, *C*, *D*). Together these facts indicated that a reaction somewhere between pH 7.0 and 5.5 was optimum for *Dictyostelium discoideum*. Further, it was shown that while a weak acid reaction favored the growth of *D. discoideum* a strong acid reaction, like an alkaline one, inhibited its growth and development.

USE OF BUFFERED AND ADJUSTED MEDIA

It was now evident that bacterial colonies of a mild acid reaction especially favored *Dictyostelium discoideum*. However, neither the range of pH within which the slime mold could grow nor the optimum pH for its growth and development was yet definitely known. In the succeeding experiments an investigation of these questions was undertaken through the use of buffered media which were adjusted to different initial reactions by the addition of N/1 hydrochloric acid or N/1 sodium hydroxide.

For the first of these experiments an agar medium containing 1.2 percent peptone was employed. A part of this medium was reserved as a control and was neither buffered nor adjusted, while the remainder was buffered with equimolar quantities of monobasic potassium phosphate and dibasic sodium phosphate and was subsequently adjusted to different pH values by the addition of N/1 hydrochloric acid. Altogether five media were prepared which were constant in nutrient but which differed one from another either in the amount of buffer they contained or in their pH adjustment (table 4).

Again *Escherichia coli* and *Pseudomonas fluorescens* were selected as bacterial associates. Since it was known that neither of these species produced colonies favoring *Dictyostelium* when grown upon peptone-rich media in the absence of buffers and pH adjustments, any growth of the slime mold that might occur upon buffered and adjusted media of like nutrient composition could be attributed to the action of the buffers in maintaining a proper pH adjustment.

Duplicate plates of the five media were inoculated with *Escherichia coli* and a like number with *Pseudomonas fluorescens* in the manner shown in figure 9. The resulting bacterial colonies were seeded with pure spores of *Dictyostelium* when 2½ days old. Incubation throughout the experiment was at room temperature (20° to 24° C.).

Dictyostelium discoideum failed to grow in cultures of either *Escherichia coli* or *Pseudomonas fluorescens* on the unbuffered and unadjusted media where the bacterial colonies became markedly alkaline in reaction; whereas it thrived in cultures of these bacteria upon buffered and adjusted media of the same nutrient content where the bacterial colonies remained acid in reaction (table 4). Thus the experiment further substantiated the writer's earlier observations that an acid reaction favored the slime mold. In addition, it clearly demonstrated that the amount of growth and the character of development of *Dictyostelium* could be in some measure correlated with the degree of acidity existing within the bacterial colonies into which it was introduced (table 4). For example, it failed to grow in bacterial colonies more alkaline than pH 8.0; grew poorly or fairly and developed abnormally in colonies of pH 7.6 to 8.0; grew well in colonies of pH 6.8

TABLE 4.—*Influence of buffer concentrations and of pH adjustment upon the growth of Dictyostelium discoideum in colonies of Escherichia coli and Pseudomonas fluorescens on media containing 1.2 percent of peptone as the sole nutrient*

Bacterial associate	Buffer used in peptone medium	Initial pH [1]	Growth of bacteria [2]	Growth of *Dictyostelium discoideum*								Final pH [4]
				Clearance of bacterial colonies	Growth of myxamoebae	Sorocarps per colony				Typical sorocarps per colony	Migration of pseudoplasmodia [3]	
						Large	Medium	Small	Medium sorocarp equivalents			
						Number	*Number*	*Number*	*Number*	*Percent*		
Escherichia coli	None	6.8–7.0	*C*	None	None	0	0	0	0	0	None	8.0–8.2
	M/150 in KH$_2$PO$_4$ and in Na$_2$HPO$_4$+12H$_2$O	6.0–6.2	*C*	Partial	Poor	1– 3	1– 3	0	9	0	Limited, up to 1 cm	7.8–8.0
	do	5.2–5.4	*C*	Nearly complete	Fair	3– 5	4– 5	3–4	19	25	do	7.6–7.8
	M/50 in KH$_2$PO$_4$ and in Na$_2$HPO$_4$+12H$_2$O	6.0–6.2	*C*	Complete	Good	25–30	30–40	15–20	134	90	Extensive, up to 3 cm	6.8–7.0
	do	5.2–5.4	*C*	do	Very good	30–35	40–50	20–25	145	90	do	6.4–6.6
Pseudomonas fluorescens.	None	6.8–7.0	*C*	None	None	0	0	0	0	0	None	8.4–8.6
	M/150 in KH$_2$PO$_4$ and in Na$_2$HPO$_4$+12H$_2$O	6.0–6.2	*C*+	do	do	0	0	0	0	0	do	8.2–8.4
	do	5.2–5.4	*C*+	Partial	Poor	0	0– 2	0	1	0	do	8.0–8.2
	M/50 in KH$_2$PO$_4$ and in Na$_2$HPO$_4$+12H$_2$O	6.0–6.2	*C*	Complete	Fair	4– 6	8–10	10–15	28	70	do	7.0–7.2
	do	5.2–5.4	*C*	do	Good	12–15	25–30	15–20	78	85	Extensive, up to 2.5 cm	6.8–7.0

[1] The pH of the medium at the time the bacteria were inoculated

[2] Growth is designated by the symbol *C* for a standard growth of *E coli* upon a medium containing 12 g of lactose and 4 g of peptone per liter (p. 165).

[3] Migration of pseudoplasmodia is recorded in the maximum distance any pseudoplasmodium migrated from the bacterial colony in which it formed

[4] The pH of the medium adjacent to the colonies of bacteria or bacteria and *Dictyostelium* at the end of the experiment

Table 5.—*Influence of hydrogen-ion concentration upon the growth of Dictyostelium discoideum in colonies of Escherichia coli and of Pseudomonas fluorescens growing upon 1.2 percent peptone agar media which was also M/50 in KH_2PO_4 and in $Na_2HPO_4+12H_2O$*

Bacterial associate	pH adjustment	Bacterial growth[1]	Growth of *Dictyostelium discoideum*							Change of pH		Migration of pseudoplasmodia[4]
			Clearance of bacterial colony	Growth of myxamoebae	Sorocarps per colony				Typical sorocarps per colony	Initial[2]	Final[3]	
					Large	Medium	Small	Medium-sorocarp equivalents				
					Number	*Number*	*Number*	*Number*	*Percent*			
Escherichia coli	3.0±	Negligible	None	None	0	0	0	0	0	3.0–3.2	3.2–3.4	None
	4.0±	*C*	Complete	Excellent	30–35	40–50	30–40	165	70	4.0–4.2	5.6–5.8	Very extensive, up to 3.5 cm
	5.0±	*C*	do	do	30–35	30–35	25–30	151	85	5.0–5.2	6.2–6.4	Extensive, up to 3 cm
	6.0±	*C*	do	Very good	20–25	20–30	20–30	108	75	6.0–6.2	6.6–6.8	Limited, up to 1.5 cm
	7.0±	*C*	Nearly complete	Good	10–12	15–20	10–15	58	50	6.8–7.0	7.2–7.4	Limited, up to 1.0 cm
	8.0±	*C*	Slight	Very poor	0	0	0	0	0	8.0–8.2	8.4–8.6	None
	9.0±	*C*	None	None	0	0	0	0	0	8.8–9.0	9.0–9.2	Do.
	([5])	*C*	Slight	Very poor	0	0	0	0	0	6.8–7.0	8.2–8.4	Do
Pseudomonas fluorescens.	3.0±	None	None	None	0	0	0	0	0	3.0–3.2	3.0–3.2	Do
	4.0±	*C*	Complete	Excellent	35–40	40–50	30–40	182	75	4.0–4.2	6.0–6.2	Very extensive, up to 4 cm
	5.0±	*C*	do	do	30–35	30–40	20–25	153	70	5.0–5.2	6.8–7.0	Extensive, up to 2.5 cm
	6.0±	*C*+	Partial	Poor	0–2	0–4	0–4	6	0	6.0–6.2	7.6–7.8	None
	7.0±	*C*+	Slight	Very poor	0	0–3	0–4	2	0	6.8–7.0	8.2–8.4	Do
	8.0±	*C*+	None	None	0	0	0	0	0	8.0–8.2	8.6–8.8	Do
	9.0±	*C*+	do	do	0	0	0	0	0	8.8–9.0	9.2–9.4	Do
	([5])	*C*+	do	do	0	0	0	0	0	6.8–7.0	8.6–8.8	Do

[1] Growth of bacteria is expressed in terms of a standard growth of *E coli* (*C*) upon a medium containing 12 g of lactose and 4 g of peptone per liter

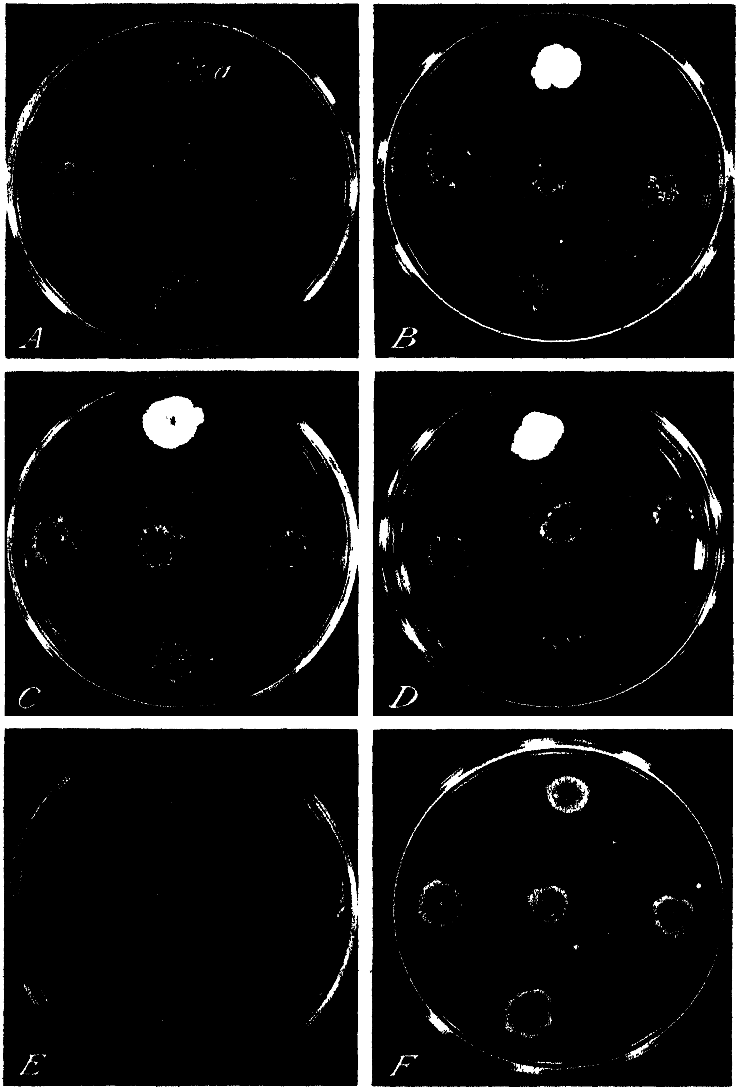
[2] Determinations made at time bacteria were inoculated upon media.

[3] Determinations made at close of experiment, readings taken from zones adjacent to colonies

[4] Recorded in maximum distance any pseudoplasmodium traveled from site of formation.

[5] Unbuffered, unadjusted.

to 7.0; and grew luxuriantly and developed in a wholly normal manner in colonies of pH 6.4 to 6.6. In addition to the correlation between the degree of acidity and the total growth of the slime mold, there also appeared to be a positive correlation between the amount of growth and the extent of pseudoplasmodial migration, and similarly between the quantity of growth and the percentage of normal sorocarps developed.



[For explanatory legend see opposite page]

The growth of *Dictyostelium* was uniformly better in cultures of *Escherichia coli* than in those of *Pseudomonas fluorescens* upon similar media, a result which was not unexpected since *Ps. fluorescens* is more actively proteolytic than *E. coli.*

A buffer concentration of M/150 in monobasic potassium phosphate (KH_2PO_4) and of M/150 in dibasic sodium phosphate ($Na_2HPO_4+12\,H_2O$) proved inadequate to maintain a favorable acid reaction in colonies of either bacterial associate upon the peptone medium employed. However, a concentration of M/50 in each of these phosphates was sufficient to maintain a mild acid reaction in colonies of the bacteria, and in such cultures *Dictyostelium discoideum* grew luxuriantly and developed normally.

Using the stronger buffer concentration (i. e., M/50 in KH_2PO_4 and in $Na_2HPO_4+12H_2O$), a second and more extensive study was now undertaken for the dual purpose of further testing the observations already made and of determining whether or not a lower pH adjustment than those previously studied would be even more favorable for *Dictyostelium discoideum.* For this investigation an agar medium containing 1.2 percent of peptone and the buffer concentration indicated above was prepared. Portions of this stock medium were then adjusted to reactions of approximately pH 3.0,[11] 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0 by the addition of N/1 hydrochloric acid or N/1 sodium hydroxide.

Duplicate plates of each medium, including an unbuffered and unadjusted one, were inoculated with *Escherichia coli* and a similar number with *Pseudomonas fluorescens.* As in the preceding experiments, these cultures were incubated at room temperature (22°–24° C.) and the resultant colonies were seeded with pure spores of *Dictyostelium* after 3 days.

A record of the growth and character of development of the slime mold in the several cultures is presented in table 5, while a graphic comparison of its growth in the different cultures is shown in figure 10.

Both bacterial associates tended strongly to shift the pH of the cultures toward an alkaline reaction (table 5) owing to the liberation of ammonia, and it is essential to recognize that the initial pH of any

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[11] To obtain a firm agar gel in strong acid media, additional agar was added up to 3 percent for the media of pH 3.0 and 2.5 percent for the media of pH 4.0, pH adjustments were made by the addition of sterile N/1 HCl after the agar media were sterilized.

EXPLANATORY LEGEND FOR FIGURE 9

FIGURE 9.—Ten-day-old cultures of *Dictyostelium discoideum* in colonies of *Escherichia coli* on buffered peptone-agar media initially adjusted to different hydrogen-ion concentrations. × ½. *A,* Initial pH 4.0±; luxuriant growth, scattered sorocarps indicate extensive migration of pseudoplasmodia; uninoculated colony (*a*) invaded by scattered myxamoebae and now indistinguishable from colonies originally inoculated; final reaction adjacent to colonies, pH 5.6–5.8. *B,* Initial pH 5.0±; slightly reduced growth and less extensive migration of pseudoplasmodia than in preceding culture; final reaction adjacent to colonies, pH 6.2–6.4. *C,* Initial pH 6.0± further reduction in the amount of growth and in the extent of pseudoplasmodial migration; final reaction adjacent to colonies, pH 6.6–6.8. *D,* Initial pH 7.0±; still further reduction in growth and in the migration of pseudoplasmodia; final reaction adjacent to colonies, pH 7.2–7.4. *E,* Initial pH 8.0±; small areas of bacterial colonies consumed by myxamoebae but no pseudoplasmodia produced; final reaction adjacent to colonies, pH 8.4–8.6. *F,* Initial pH 9.0±; no growth of *Dictyostelium,* the spores remaining ungerminated; final reaction adjacent to colonies, pH 9.0–9.2. (Photographs by Frank White.)

medium was of importance only insofar as it influenced the growth of the host bacteria and the pH of the bacterial colonies during the growth period of the slime mold. Determinations of the pH of bacterial colonies were not made at the time of inoculation with *Dictyostelium*, but since the bacteria had almost if not wholly ceased growing by that time, it is believed that the determinations made at the close of the experiment fairly represent the pH of the bacterial colonies throughout the whole period of slime mold growth.

The growth of *Dictyostelium discoideum* could be closely correlated with the hydrogen-ion concentration of the host bacterial colonies (table 5). Spores of *Dictyostelium* failed to germinate in colonies with a final reaction in excess of pH 8.5, and myxamoebae grew very poorly or not at all in colonies with a reaction of pH 8.0 or above. Poor growth of myxamoebae occurred and abnormal sorocarps were developed, but no migration of pseudoplasmodia took place from colonies with a final reaction between pH 7.5 and 8.0; fair to good growth of *Dictyostelium* occurred in colonies of pH 7.0 to 7.4, but a large percentage of the sorocarps produced were of abnormal pattern; whereas very good to excellent growth and wholly normal development of *D. discoideum* occurred in colonies of bacteria in which the final reaction was between pH 5.0 and 7.0. The optimum reaction appeared to be in the neighborhood of pH 6.0.

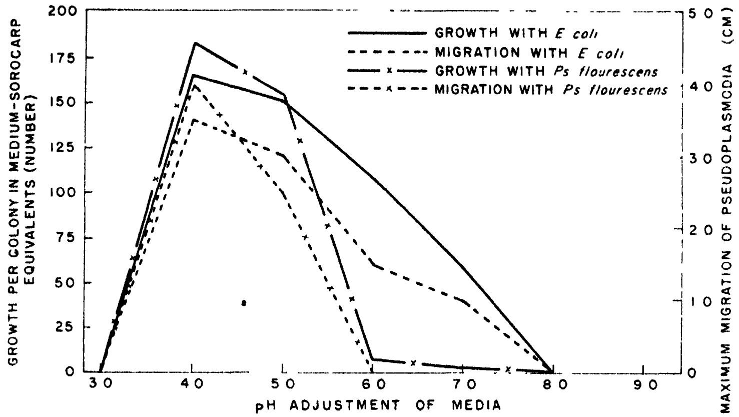

FIGURE 10.—Comparative growth and pseudoplasmodial migration of *Dictyostelium discoideum* in association with *Escherichia coli* and with *Pseudomonas fluorescens* upon buffered peptone-agar media of different initial pH adjustments.

Neither *Escherichia coli* nor *Pseudomonas fluorescens* grew upon media adjusted to pH 3.0; and since there were no bacteria present *Dictyostelium discoideum* could not grow for lack of food, quite aside from any effect the strong acid reaction in itself might have had upon its growth and development.

The percentage of typical sorocarps could in general be correlated with the amount of growth (table 5). That is, a bacterial colony that favored a luxuriant growth of *Dictyostelium* also favored the production of a large percentage of normal sorocarps. Also the ratio of normal to abnormal sorocarps was regularly much greater among sorocarps resulting from migrating pseudoplasmodia than among sorocarps produced from nonmigrating pseudoplasmodia. This was to be expected, for it could be assumed that the more vigorous pseudoplasmodia would migrate and that they also would produce sorocarps of the pattern most characteristic of the species. The extent of migration of pseudoplasmodia could likewise be correlated with the favorableness of the culture. That is, where the greatest growth and most nearly normal development of *Dictyostelium* occurred, there also occurred the greatest migration of pseudoplasmodia, not only in the number migrating but also in the maximum distances traveled by individual pseudoplasmodia (fig. 10).

Paralleling the study described above, a similar experiment was conducted in which a stock medium containing 1.2 percent each of peptone and sucrose was substituted for the one containing peptone alone. This yielded results essentially like those obtained with the peptone media except that with both *Escherichia coli* and *Pseudomonas fluorescens* maximum growth of *Dictyostelium discoideum* occurred upon media initially adjusted to pH 5.0 instead of 4.0. As with the cultures upon peptone media, however, maximum growth of the slime mold occurred in bacterial colonies with a final reaction of approximately pH 6.0, while the percentage of normal sorocarps and the extent of pseudoplasmodial migration could again be correlated with the total growth of the slime mold.

Thus it was demonstrated that the growth of *Dictyostelium discoideum* is governed by the hydrogen-ion concentration of the bacterial colonies into which it is introduced, and that a reaction of pH 6.0± is apparently optimum for its growth and normal development. Furthermore, it was shown that such characters of the fruiting stage as pseudoplasmodial migration and sorocarp pattern can be used as an index to the favorableness of a culture.

INCUBATION TEMPERATURE OF BACTERIA PRIOR TO THE INTRODUCTION OF DICTYOSTELIUM

During the course of previous investigations it was occasionally, though rarely, noted that *Dictyostelium discoideum* grew poorly and irregularly in colonies of *Escherichia coli* upon media containing 1.2 percent each of peptone and lactose. This was indeed puzzling because excellent growth normally occurred in colonies of this bacterium upon media of the above-mentioned composition. A possible clue was obtained when the pH of such cultures was found to be uniformly of a strong acid reaction, ranging from pH 4.0 to 4.5. Certain possible explanations of this phenomenon presented themselves; but the temperature at which the bacterial cultures were incubated prior to inoculation with *Dictyostelium* offered the most promising lead, for it was observed that in all cases such cultures had been exposed for longer or shorter periods to temperatures somewhat in excess of the normal temperature of the laboratory.

In order to investigate this lead, therefore, plates of agar media containing 1.2 percent each of peptone and lactose were inoculated with *Escherichia coli* and incubated for 3 days, some at 35° C., some at 25° to 26°, and others at 15°. Pure spores of *Dictyostelium* were introduced into the bacterial colonies after 3 days, and all of the cultures were subsequently incubated at the uniform temperature of 22° to 23°.

TABLE 6.—*Growth of Dictyostelium discoideum in colonies of Escherichia coli grown upon lactose-peptone media at different temperatures for 2 days prior to the introduction of Dictyostelium discoideum spores*

Incubation temperature of bacteria (° C.)	Bacterial growth [1]	Growth of *Dictyostelium discoideum*								pH		
		Clearance of bacterial colony	Growth of myxamoebae	Sorocarps per colony				Typical sorocarps per colony	Migration of pseudoplasmodia	Initial pH of medium	Final pH[2] in colonies	Final pH between colonies
				Large	Medium	Small	Medium-sorocarp equivalents per colony					
				Number	*Number*	*Number*	*Number*	*Percent*				
35°	*C*	Partial	Poor	0	0	0	0	0	None	6.8-7.0	4.0-4.2	4.4-4.6
25°-26°	*C+*	Nearly complete	Good	8-10	10-15	6-10	45	55	Up to 2.5 cm	6.8-7.0	4.4-4.6	5.3-5.5
15°	*C*	Complete	Very good	25-30	25-30	20-30	130	75	Up to 4.5 cm	6.8-7.0	6.6-6.8	6.4-6.6

[1] Growth is expressed in terms of a standard growth of *E. coli* (*C*) upon a medium containing 12 g of lactose and 4 g of peptone per liter.

[2] Determinations made upon agar surface immediately adjacent to the bacterial colonies

The results of the experiment, which are shown in table 6, are self-explanatory, but the following points are worthy of special attention: (1) The final reaction of bacterial colonies in cultures initially incubated at 35° C. was strongly acid, pH 4.0 to 4.2, and the growth of *Dictyostelium* was extremely poor and its development very abnormal. (2) The final reaction of colonies initially incubated at 25° to 26° was slightly less acid, pH 4.4 to 4.6, and although fair growth of the slime mold occurred, its development was abnormal in character. (3) The final reaction of bacterial colonies initially incubated at 15° was near neutral, pH 6.6 to 6.8, and the growth of *Dictyostelium* was very good and its development was essentially normal.

A similar and even more striking experiment confirming the above results was readily performed, Endo's differential medium being used. Colonies of *Escherichia coli* upon this medium when incubated at 35° C. became deep red in color, flat, dryish, and were characterized by a metallic sheen. The underlying medium also became a deep red, indicating a strong acid reaction. In contrast, colonies incubated at a temperature of 22° to 23°, or less, remained light pink in color, raised, moist, and devoid of sheen, and the underlying medium became light red, indicating a mild acid reaction. In the latter and weakly acid colonies *Dictyostelium discoideum* grew luxuriantly and developed normally, whereas it failed to grow in the former strongly acid cultures.

Thus in these experiments, as in previous ones, the growth and development of *Dictyostelium discoideum* were governed by the hydrogen-ion concentration of the bacterial colonies into which it was introduced. However, in these latter cases, the inhibition of growth and the production of abnormal fruiting structures were due to a too acid rather than a too alkaline reaction.

USE OF NITROGEN SOURCES OTHER THAN PEPTONE

Peptone was employed almost exclusively as a source of nitrogen in the present studies. However, this nutrient is not essential for the successful cultivation of *Dictyostelium discoideum* on artificial media, and other nitrogen sources, including asparagine, ammonium chloride, ammonium nitrate, and ammonium phosphate, were successfully employed in conjunction with fermentable carbohydrates. In cultures with these nitrogen sources, as in those with peptone, a definite relationship existed between the hydrogen-ion concentration of the bacterial colonies and the subsequent growth and development of *D. discoideum*. The slime mold grew poorly or not at all in bacterial colonies more alkaline than pH 8.0; whereas in colonies of pH 4 8 to 7.0 sorocarp development was for the most part normal, indicating that conditions were essentially favorable for *D. discoideum*.

STUDIES WITH NATURAL MEDIA

Following the completion of the studies with peptone-sugar media, further experimentation with natural substrata supplied additional proof of the importance of the hydrogen-ion concentration of the host bacterial colonies. In connection with preliminary studies (p. 160) it was noted that carrot-infusion agar afforded a reasonably satisfactory medium for the cultivation of *Dictyostelium discoideum* in association with *Pseudomonas fluorescens* or *Serratia marcescens;* whereas yeast and bean-infusion agar, although supporting an ample growth of bacteria, did not permit *D. discoideum* to grow and develop normally. Therefore, it is of interest to note that the carrot-infusion agar was mildly acid in reaction at the time of inoculation with bacteria and remained so throughout the growth period of the bacteria and subsequently of the slime mold; while, on the contrary, the yeast and bean-infusion agars, although near neutral in reaction when inoculated with bacteria, became quite alkaline during the growth of the bacteria, and the growth of the slime mold was subsequently inhibited.

Pinoy (*16*) reported that *Dictyostelium mucoroides* in association with *Bacillus fluorescens liquefaciens* thrived upon pieces of sterilized carrot but could not grow with this same bacterial associate upon pieces of sterilized potato, although abundant bacteria were present for the slime mold to feed upon. Skupienski (*24*) later confirmed Pinoy's results and attributed the difference in behavior to the presence in the one medium of some essential substance that was absent from the other. The writer, however, believes that the presence of growth upon carrot and its absence upon potato were due to a difference in the hydrogen-ion concentration of the bacterial colonies upon the two substrata. As already noted, carrots and carrot agar are of a mild acid reaction and the bacterial colonies developing upon these media remain acid in reaction, and in them *D. discoideum* grows well and develops in a normal manner. On the other hand, although sterilized potato and potato-infusion agar are initially of a weak acid reaction, upon these media colonies of *Pseudomonas fluorescens* (and *Serratia marcescens* likewise) become markedly alkaline in reaction (pH 8.0–8.2 ±) and *D. discoideum* fails to grow in them. Thus it would seem that the presence of good growth of *Dictyostelium* upon sterilized carrot and its absence upon sterilized potato can be attributed to the difference in hydrogen-ion concentration of the bacterial colonies upon the two substrata.

It is of interest to call attention to the striking similarity of results obtained by the writer with *Dictyostelium discoideum* in association with *Serratia marcescens* and by Cutler and Crump with a soil amoeba, *Hartmanella hyalina* Dangeard, in association with the same bacterial species. Cutler and Crump in 1927 (*5*) reported that *H. hyalina* could not feed upon *Bacillus prodigiosus* Flügge (*S. marcescens*), but in 1935 (*6*) they observed that the amoeba could grow with this bacterium in soil extract and pointed out that in their earlier work they had attempted to grow the two organisms upon nutrient agar. In the present study the writer found that *D. discoideum* could not grow with *S. marcescens* upon nutrient agar but that it grew well with this bacterium upon hay-infusion agar (*19*), a medium more or less comparable in nutritive value to the soil extract employed by Cutler and Crump. Upon nutrient agar the bacterial colonies became strongly alkaline, while upon hay-infusion agar they remained slightly acid or became neutral in reaction.

The writer is not prepared to say what role the hydrogen-ion concentration of substrata and of host bacterial colonies plays in governing the growth of soil amoebae generally, but the case at hand is suggestive and is of particular interest because of the remarkable parallelism of behavior shown.

DISCUSSION

In this study the formation or nonformation of sorocarps has been considered the primary measure of the favorableness or unfavorableness of cultures to *Dictyostelium discoideum*, while the form or pattern of the sorocarps produced has been interpreted as indicating the degree of favorableness of cultures. This practice is based upon consistent results obtained by the writer from repeated experiments, and although it was not employed by earlier students of the Dictyosteliaceae, it is wholly reliable and affords a most convenient means of evaluating the worth of different combinations of culture media and bacterial associates.

In *Dictyostelium discoideum*, as in all the Dictyosteliaceae, the vegetative phase and the fruiting phase are clearly set apart; and from the results of the current studies it is plainly evident that these two phenomena are not influenced, retarded, or inhibited to the same extent by the presence of toxic conditions within the colonies of host bacteria. Degrees of inhibition can be readily distinguished and these can be correlated with the nutrient content of the media and the fermentations of the associated bacteria (tables 1 and 2), and likewise with the hydrogen-ion concentration of the host bacterial colonies (tables 3, 4, 5, and 6). In bacterial colonies more alkaline than pH 8.6, the spores of *D. discoideum* fail to germinate; in colonies of pH 8.2 to 8.6, limited myxamoebic growth normally occurs and an occasional pseudoplasmodium forms but no sorocarps develop; in colonies of pH 7.5 to 8.0, fair growth of the slime mold ordinarily takes place, limited numbers of pseudoplasmodia form, and some sorocarps develop but the majority of these are of abnormal pattern (fig. 3, *E–I*); while in colonies of pH 5.0 to 7.0, excellent growth of *Dictyostelium* invariably occurs, numerous pseudoplasmodia form, and the great majority of these subsequently develop into sorocarps of normal form and proportions (fig. 3, *A–C*).

In colonies more acid than pH 5.0 there is likewise a steady reduction in the amount of growth accompanied by an increasingly abnormal development of the slime mold as the acidity increases. However, the limiting influence of acidity has not been adequately investigated as yet, and reactions at which different degrees of inhibition occur cannot be given.

Further consideration of the significance of sorocarp patterns is believed worth while. The process of sorocarp formation is delicately sensitive to culture conditions, and slight differences in environment are rendered apparent during the formation of fruiting structures. Under optimum culture conditions, the host bacterial colonies are completely consumed and all of the myxamoebae, with the exception of scattered individuals, aggregate into pseudoplasmodia which subsequently resolve into sorocarps of normal pattern that are entirely typical for the species; whereas, under less favorable conditions the bacterial colonies remain incompletely consumed, a large proportion of the myxamoebae never aggregate into pseudoplasmodia, and the sorocarps formed become increasingly less normal in pattern as conditions become more unfavorable until finally they cease to be formed altogether.

The first indication of unfavorable culture conditions, as reflected in sorocarp pattern, appears in the stalk, or sorophore, and basal disk.

The stalk is shorter, thicker, and more strongly tapered than in typical structures, while the basal disk is less expanded and more conical in shape and tends to merge imperceptibly into the base of the stalk. In such mildly abnormal sorocarps (fig. 3, *D* and *E*) the sorus appears relatively large and out of proportion to the size of the fruiting structure as a whole. As conditions become more unfavorable, the stalk becomes even shorter and thicker and the basal disk less apparent, while the sorus loses its characteristic form and appears to blend into the supporting stalk (fig. 3, *F*) in the manner of a fruiting structure of *Guttulina* (*14*). Under still less favorable conditions the myxamoebae become heaped up into a mass in which there is no differentiation either into fertile spores or characteristic stalk cells (fig. 3, *G*), the whole structure superficially resembling a fruiting mass of the simple genus *Guttulinopsis* (*14*). Finally under the least favorable conditions where growth of *D. discoideum* is possible, the myxamoebae grow meagerly and consume a limited portion of the host bacterial colony but are subsequently incapable of aggregating into pseudoplasmodia.

During the present study considerable evidence has accumulated indicating that either incubation at a temperature somewhat in excess of the optimum for this species or exposure for brief periods to the unsaturated air of the laboratory will bring about the formation of abnormal sorocarps in cultures characterized by a favorable hydrogen-ion concentration. However, in the experiments discussed herein these factors have not been of importance, since in all cases the cultures were incubated at temperatures favorable to *Dictyostelium discoideum* (18° to 24° C.), and in all cases the cultures were grown in closed Petri plates consisting of close-fitting valves that insured the maintenance of a saturated or near-saturated atmosphere within the cultures.

In the writer's experience the following factors are of primary importance in governing the growth and development of *Dictyostelium discoideum:* (1) The composition of the culture medium, (2) the fermentations of the associated bacteria, (3) the hydrogen-ion concentration of the host bacterial colonies, and (4) the physical character of the bacterial growth.

Combinations of these factors operate in any given culture to determine whether or not good growth and normal development of the slime mold can take place; and in no case is it possible to consider either the first or the second of these factors apart from the other, for it is only through one that the other becomes operative. *Dictyostelium discoideum* grows luxuriantly and develops normally at room temperature in colonies of bacteria upon media containing 1.2 percent peptone and an equal quantity of some carbohydrate that the associated bacteria can ferment; whereas it grows very poorly or not at all in colonies of the same bacteria upon media containing a like quantity of peptone as the sole nutrient, or combined with an unavailable carbohydrate (fig. 4). Clearly then, upon peptone-rich media, good growth and normal development of *D. discoideum* can be attributed to the presence of a carbohydrate that can be utilized by the associated bacteria. Peptone, or some other nitrogen-rich nutrient, cannot be omitted from such a medium, for in its absence the associated bacteria grow sparingly and the growth of the slime mold is limited accordingly. On the other hand, an available carbohydrate cannot be omitted, for, although its presence does not materially enhance the growth of host bacteria (table 1), it insures that the slime mold can feed upon the bacteria that are present.

The importance of the nutrients used and of the fermentations by the host bacteria becomes progressively lessened as media containing successively lower concentrations of nutrients are employed. Upon media containing 1.2 percent peptone as the sole nutrient, the growth of *Dictyostelium* is very slight or wholly lacking; whereas upon media containing 0.1 percent peptone, although the growth of the slime mold is restricted in amount, owing to the limited growth of host bacteria, colonies of bacteria are wholly consumed and sorocarps of essentially normal pattern are produced (table 1).

The hydrogen-ion concentration of the host bacterial colonies is, in the writer's experience, the most important factor governing the growth and development of *Dictyostelium discoideum*. And, indeed, it is primarily through their influence upon the pH of the colonies of associated bacteria that the above factors of medium composition and bacterial fermentations operate. Optimum growth and development of *D. discoideum* occur in bacterial colonies of approximately pH 6.0, while good growth and normal development regularly occur in colonies having a reaction between pH 5.0 and 7.0. Outside of this favorable pH range, the growth of the slime mold becomes progressively less in amount and its development increasingly more abnormal as the colonies of host bacteria become either more alkaline or more acid. As demonstrated by repeated experiments, the manner in which a favorable reaction is established in the bacterial colonies is relatively unimportant; and it matters not whether it is produced by the simultaneous and balanced fermentation of some nitrogen source and some carbohydrate by the associated bacteria themselves (*1*), or by some artificial means such as the addition of free acid or the use of buffers and pH adjustments.

The results obtained from the studies with Endo's and allied media (fig. 2) and with the peptone-sugar media (table 1) can be fully explained on the basis of the hydrogen-ion concentration of the bacterial colonies, although pH determinations were not made in these early experiments. In these studies it was uniformly found that the presence or absence of good growth and normal development of *Dictyostelium discoideum* with any particular bacterial host could be correlated with the ability or inability of that bacterium to ferment the carbohydrate contained in the medium (figs. 2 and 4). And in later experiments where pH determinations were consistently made it was found that the fermentation or nonfermentation of the carbohydrate was invariably expressed in the pH of the bacterial colonies. If the carbohydrate was not fermented and only the peptone was broken down the bacterial colonies became alkaline in reaction (pH 8.4–8.6) and *Dictyostelium* failed to grow; whereas, if both the carbohydrate and the peptone were fermented the bacterial colonies became mildly acid in reaction (pH 5.5–7.0) and the slime mold grew luxuriantly and developed normally. At the same time it should be recalled that bacterial colonies of a strong acid reaction are just as toxic to *D. discoideum* as those of a marked alkaline reaction (p. 187) and when culture conditions are such that the acid products of carbohydrate fermentation outweigh the alkaline products of peptone decomposition, the bacterial colonies become strongly acid and the growth of

Dictyostelium is inhibited. Thus, if either process of decomposition overbalances the other, *Dictyostelium discoideum* is unable to grow in the resulting bacterial colonies, on the one hand because they are too alkaline in reaction (pH 8.5 and above) and on the other because they are too acid (pH 4.0 and below).

Finally, not only must the composition of the medium, the fermentations by the bacteria, and the hydrogen-ion concentration of the bacterial colonies be taken into account in order to cultivate *Dictyostelium discoideum* successfully upon peptone-sugar media, but the physical character of the bacterial growth must also be such as to favor the feeding myxamoebae. To illustrate, *Achromobacter radiobacter* (Beijerinck and Van Delden) Bergey et al., *Aerobacter aerogenes* (Kruse) Beijerinck, and *Phytomonas tumefaciens* (Smith and Townsend) Bergey et al. afford excellent nutriment for *D. discoideum* when grown upon a suitable medium such as hay-infusion agar (*19*); and among other carbohydrates each bacterium can ferment dextrose and sucrose. However, when these bacteria are grown upon media containing 1.2 percent each of peptone and dextrose or peptone and sucrose, large lens-shaped colonies of a gummy character are produced, and in these colonies the slime mold grows sparingly because of the inability of the myxamoebae to feed effectively upon the gum-encased bacterial cells. No toxic condition exists within such colonies, for the sorocarps produced, although few in number and for the most part small in size, are normal in pattern; while throughout the experiments reported in this paper it was consistently found that cultures in which toxic conditions existed, whether due to alkalinity or excess acidity, were characterized either by abnormal sorocarps or by a complete lack of fruiting structures.

Although in the writer's experience the factors just considered truly govern the growth and development of *Dictyostelium discoideum*, caution should be exercised against making too great generalizations concerning them. Different strains of the same bacterial species may vary in the physical character of their growth, while they may likewise differ appreciably in their ability to break down peptone and to ferment particular sugars; consequently, the concentrations of nutrients employed may need to be varied accordingly. Furthermore, peptones vary appreciably in character (e. g., acidity) and in nutritive value (p. 161), and such variations must also be taken into account. Thus any new combination involving either an untried strain of host bacteria or an unfamiliar medium may present new problems. However, repeated experiments have demonstrated that, so long as the hydrogen-ion concentration of the host bacterial colonies falls between pH 5.0 and 7.0 and so long as the bacterial growth is of a favorable physical character, good growth and normal development of *Dictyostelium discoideum* may be expected. Obviously, then, for the successful cultivation of this slime mold upon media rich in nutrients one should habitually employ combinations of media and bacterial associates which will yield nongummy colonies and which will enable a balanced fermentation of nutrients to take place, thereby insuring a favorable reaction in the colonies of host bacteria. Or, failing in this, one should incorporate sufficient buffers into the medium to maintain a suitable reaction regardless of the fermentative activities of the associated bacteria.

The results obtained by the writer with media containing peptone agree only in part with the findings of earlier investigators[12] who employed this nutrient. Nadson (*12*) was the first to use peptone for the cultivation of a species of *Dictyostelium* and was likewise the first to attribute a significant role to the reaction of the culture medium. He contended that the associated bacteria (*Bacillus fluorescens liquefaciens*) favored the growth of *D. mucoroides* by creating an alkaline environment. Contrary to his report for *D. mucoroides*, the writer has found that an alkaline reaction inhibits rather than favors the growth of *D. discoideum*. Olive (*14*) likewise reported successfully cultivating members of the Dictyosteliaceae upon a peptone-agar medium. However, he did not know the identity of the bacteria associated with his cultures, nor did he state the concentration of peptone employed; therefore, a close comparison between his and the writer's work cannot be made. In the event that he employed a weak concentration of peptone, the growth of the slime molds can be readily understood; for, in the writer's experience, *D. discoideum* will grow and develop more or less normally in bacterial colonies upon media containing 0.1 or 0.2 percent of peptone as the sole nutrient, although it grows poorly or not at all upon media containing higher concentrations of peptone unless this nutrient is accompanied by adequate buffers or by some carbohydrate that is fermentable by the associated bacteria.

The work by Potts (*17*) constitutes the most thorough physiological study previously made on a species of the Dictyosteliaceae. He was fully aware that, in general, sources of nitrogen yield alkaline products, whereas sources of carbon yield acid products when decomposed by bacteria. He was not unmindful of the importance of the nutrient employed, nor of the possible influence the associated bacteria might wield through altering the culture medium. Furthermore, he observed that the growth of *Dictyostelium mucoroides* was dependent on a favorable balance between these opposing products, but he did not consider the reaction of the medium as the chief factor influencing the growth of the slime mold. He reported that *D. mucoroides* grew well in cultures ranging from weakly acid to strongly alkaline, and reached its maximum growth in weakly alkaline cultures. This he determined by growing *D. mucoroides* in association with *Bacterium fimbriatum* in liquid media (presumably maize extract) containing different quantities of sodium carbonate. Contrary to the above conclusion, the results listed in tables 1 and 2 of Potts' paper show that the reaction was uniformly acid or weakly acid in cultures where the best growth of *Dictyostelium* occurred. From this conflicting evidence the writer feels justified in accepting Potts' results with many different media, as listed in his tables, in preference to the result obtained by him with a single medium in which the concentration of sodium carbonate was used as an indicator of alkalinity.

Vuillemin (*27*) successfully cultivated *Dictyostelium mucoroides* in association with *Bacillus fluorescens non-liquefaciens* upon a maltose-

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[12] It should be noted that earlier investigators in all cases worked with species of *Dictyostelium* other than the one studied by the writer, and in the majority of cases with the ubiquitous species *D. mucoroides*. Nonetheless, the writer feels that in several instances a comparison of the results obtained by himself and those obtained by earlier investigators can be made, since *D. mucoroides* frequently occurs in and readily may be isolated from decaying vegetation not unlike that from which *D. discoideum* was obtained (*18*), and since the writer, in experimental studies carried on along with this work but as yet unpublished, found that conditions which favor the growth of *D. discoideum* also favor that of *D. mucoroides*.

(2.0 percent) peptone (0.5 percent) medium, but he could not grow the slime mold in association with *Bacillus pyocyaneus* Gessard upon the same medium. Knowing that *B. pyocyaneus* was an especially active ammonifier, he interpreted this result as contradicting Nadson's view (*12*) that *D. mucoroides* was favored by an alkaline reaction, a criticism which the writer's work with *D. discoideum* indicates was justified. On the other hand, since *B. fluorescens non-liquefaciens* cannot ferment maltose (*27*), the explanation of *Dictyostelium* growth with this species is not at first obvious. However, when both the weak proteolytic character of the bacterium, as evidenced by the nonliquefaction of gelatin, and the relatively low concentration of peptone are taken into account, Vuillemin's success can in large measure be explained.

Pinoy (*16*) reported that *Dictyostelium mucoroides* could not grow in pure-mixed culture with most bacteria upon bouillon or petone agars unless sugar ("glucose, lactose, maltose") were added. Obviously he had made sufficient culture studies to secure a hint as to the relation between culture-medium composition and the growth of *Dictyostelium.* However, he did not record the identity of the bacteria to which he referred; he did not state the concentrations of nutrients used; and he considered together three sugars, each of which imparts distinct properties to a culture medium. Since either of the above factors, when uncontrolled, is capable of altering the cultural picture completely, it is impossible to draw close parallels with the results of the writer's work. Concerning the influence of the reaction of the culture upon the growth and development of the slime mold, he reported that the fruiting structures of *D. mucoroides* in pure-mixed culture with *Bacillus fluorescens liquefaciens* varied in color with the reaction of the culture, being yellow in cultures of alkaline reaction and white in those of acid reaction. But aside from this observation, he apparently did not consider the reaction of the culture of importance in influencing the growth or development of *D. mucoroides.*

Schuckmann (*21*, *22*) cultivated *D. mucoroides* upon a weak beef-infusion agar,[13] a medium not essentially different from a weak peptone agar. His success, the writer believes, was due to the dilute nutrient employed. In this connection, however, it is interesting to note that upon this medium his *Dictyostelium* progressively lost its ability to form fruiting structures (a capacity which could be restored by reculturing it upon horse dung) although the myxamoebae grew well and pseudoplasmodia were regularly formed (*21*). It would seem possible, therefore, that he encountered a culture condition sufficiently toxic to inhibit the formation of sorocarps but not the growth and aggregation of myxamoebae, a condition not unlike that which the writer reports for *D. discoideum* when grown upon media containing moderate amounts of peptone in the absence of sugars which are available to the associated bacteria (e. g., *D. discoideum* in association with *Pseudomonas fluorescens* on S4 medium, table 1).

Brefeld (*3*, *4*), Van Tieghem (*25*, *26*), Olive (*14*), and Harper (*8*, *9*, *10*) did not concern themselves with either the bacterial species that accompanied their cultures or with the reaction of the media they employed. They could neglect these factors and still secure cultures of the Dictyosteliaceae because they employed media which contained nutrients that were not readily broken down into alkaline or acid products by the bacteria that accompanied their cultures. In an earlier study the writer (*19*) demonstrated that *Dictyostelium discoideum* could grow and develop normally in association with many different bacterial species when cultivated upon hay-infusion agar, a medium poor in accessible nutrients; and in reporting this study the writer stated the following general rule concerning the relationships between the composition of the culture medium and the growth of a member of the Dictyosteliaceae (*19*, *p. 312*):

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[13] The medium employed was a so-called amoeba-agar, containing 2 percent agar in 10 parts of beef infusion and 90 parts of distilled water, which had been successfully used by Wasielewski and Kühn (*28*) for cultivating small amoebae belonging to the genus *Valkampfia.*

> The more dilute a medium is in nutrients, or the less accessible its nutrients are to a large number of different bacterial species, the more probable it is that a member of the Dictyosteliaceae will be able to grow in association with a large number of bacterial species upon that medium.

The correctness of this observation is further confirmed by the results of the present study, for it has been found that *D. discoideum* can grow and develop in an essentially normal manner upon media containing a low concentration of some nutrient, such as peptone, when it grows poorly or not at all in company with the same bacterial associates upon media containing higher concentrations of the same nutrient (table 1).

What, then, is the advantage in employing nutrient-rich media? It is solely for the purpose of securing a more luxuriant growth of the slime mold. And since *Dictyostelium* feeds upon bacteria directly, the primary requisite for a luxuriant growth is a correspondingly abundant food supply. Secondary requisites are, of course, (1) that the colonies of bacteria possess a favorable hydrogen-ion concentration, (2) that the bacterial cells be free from an excess amount of gum, (3) that the cultures be incubated at a favorable temperature, and (4) that a proper degree of humidity be maintained within the culture chambers. Obviously, in order to secure a rich growth of bacteria, the culture medium must contain an abundant supply of nutrients that are readily accessible to the bacteria. At the same time, the more accessible the nutrients are, the more rapidly toxic byproducts will be liberated by the bacteria, and consequently the more carefully the nutrients must be balanced in order that their decomposition products will neutralize each other and not accumulate in quantities detrimental to the slime mold. The use of buffers and pH adjustments can, of course, be substituted for a balance between the carbon and nitrogen sources, but the final aim remains the same, i. e., to prevent the accumulation of decomposition products which in sufficient quantity are toxic to *Dictyostelium*.

SUMMARY

Agar media excellently suited for the laboratory cultivation of *Dictyostelium discoideum* were prepared with peptone and a suitable carbohydrate as nutrients.

A medium containing 1.2 percent each of peptone and a carbohydrate that could be fermented by the associated bacteria provided an especially favorable substratum for the growth of host bacteria and for the subsequent growth and normal development of *Dictyostelium*.

The growth and development of the slime mold upon such media depended upon the composition of the culture medium and the fermentative powers of the associated bacteria.

The growth of the associated bacteria was roughly proportional to the concentration of peptone employed. The incorporation of an available carbohydrate did not enhance the growth of bacteria as a rule, but did insure that the bacterial colonies would be nontoxic to the slime mold. Colonies of host bacteria in the absence of a fermentable carbohydrate became alkaline in reaction because of the accumulation of ammonia liberated from the peptone, and were toxic to *Dictyostelium discoideum*. Colonies of the same bacterial species in the presence of a fermentable carbohydrate in addition to the peptone remained neutral or became mildly acid in reaction because of the neutralization of ammonia by acid products simultaneously produced from the carbohydrate, and were nontoxic to *D. discoideum*.

The growth and development of *Dictyostelium discoideum* were governed by the hydrogen-ion concentration of the bacterial colonies into which the slime mold was introduced. The optimum reaction for the species was pH 6.0 to 6.5, while excellent growth and the formation of normal fruiting structures, or sorocarps, regularly occurred in bacterial colonies of pH 5.0 to 7.0. Outside of this favorable range the growth of the slime mold decreased progressively and its development became consistently more abnormal as the colonies of host bacteria became either increasingly alkaline or more acid in reaction. All growth of the slime mold ceased on the one hand at pH 8.5 to 8.6 and on the other at pH 4.0 to 4.2

The method by which a favorable hydrogen-ion concentration was established in the colonies of host bacteria was apparently unimportant and could be accomplished by the simultaneous and balanced fermentation of both carbon and nitrogen sources by the associated bacteria themselves, or by the addition of free acid to cultures of alkaline reaction, or by the incorporation of sufficient buffers into the media to maintain a favorable reaction regardless of the fermentations by the associated bacteria.

The buffer concentration necessary to maintain a suitable reaction in the colonies of host bacteria depended upon the nutrient composition of the medium, the fermentative capacity of the selected bacterial associate, and the initial pH of the culture medium. Thus peptone media intended for the cultivation of *Dictyostelium discoideum* with *Pseudomonas fluorescens* required either a stronger buffer concentration or a lower initial pH adjustment than media intended for use with *Escherichia coli,* because the former species is the more strongly proteolytic.

In addition to a favorable concentration of hydrogen ions, colonies of host bacteria must have a favorable physical character in order for *Dictyostelium discoideum* to thrive. The myxamoebae could not feed effectively upon bacterial cells encased in gum or slime, although the colonies in which these occurred were wholly nontoxic to the slime mold.

A more nearly optimum cultural environment was required for the organization of myxamoebae into fruiting structures than for their vegetative growth, and in turn a more nearly optimum environment was required for the formation of normal than abnormal sorocarps. *Dictyostelium discoideum* was found to be very sensitive to culture conditions, differences in environment being reflected in the pattern of the sorocarps produced. At the same time, the pattern of sorocarps served as a useful gage of the favorableness of cultures to this slime mold. Thus *D. discoideum* affords a particularly useful species for investigating the influence of environmental conditions upon the growth and behavior of an amoeboid organism of the soil.

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RELATION BETWEEN CONFORMATION AND ANATOMY OF COWS OF UNKNOWN PRODUCING ABILITY[1]

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PLAN OF THE INVESTIGATION

The subject of body conformation in cattle is of much practical importance, for body form or so-called type to a very large extent provides the basis for selecting individuals for purchase or sale, for mating, for producing purposes, and for judging individuals in the show ring.

Some years ago a plan was evolved to determine such interrelationships as may exist between a cow's conformation, anatomy, and ability to produce milk and butterfat. Conformation in this study refers to the size, shape, and proportions of the outside of the animal body, whereas internal anatomy refers to the size, shape, and proportions of the body cavities and to the size of internal organs as indicated by their weight and measurements.

The problem consists of two main parts. The first is a consideration of the relationship between conformation and internal anatomy. It necessitates measuring the external dimensions of cows in definite units thereby translating conformation into numerical values for purposes of tabulation and analysis, and slaughtering the cows and making a detailed post-mortem study of their internal anatomy. The second part is a study of the relation between both the conformation and the internal anatomy of cows and their producing capacity as previously measured by records of milk and butterfat production.

Arrangements were made with Swift & Co. to conduct in their Chicago packing house a study of the conformation and anatomy of a large number of cows. Since the production records of these animals were not available, the present report deals only with the first part of the problem. Work on the second part is in progress with cows of known producing capacity.

HISTORY OF WORK BEARING ON THE INVESTIGATION

Mierswa (*8*)[3] has reviewed much of the literature pertaining to the relation between body form, anatomy, and performance of farm animals which appeared between 1805 and 1929, and his discussion has been supplemented by that of Becker (*1*) who reviewed the literature published between 1852 and 1911.

Baudement (*8*) found that in oxen of similar age and weight the weight of the lungs decreased as the chest circumference increased.

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[2] The writers are indebted to Swift & Co., Chicago, for providing the animals and the personnel and equipment of their plant which made this study possible; and to the Meat Inspection Division of the Bureau of Animal Industry, U. S. Department of Agriculture, for assistance in obtaining the post-mortem data.

[3] Italic numbers in parentheses refer to Literature Cited, p. 234.

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His studies with cattle (*8*) showed also some positive relation between size of lungs and height at withers, larger lungs in young than in older animals of the same live weight, a negative correlation between lung weight and live weight in animals of the same breed, and smaller lungs—absolutely and relatively—in early- than in late-maturing breeds. He concluded that the respiratory activity is smaller in animals showing a larger gain in meat. Considerable importance is attached to his finding that the size of lungs is different in early- than in late-maturing animals and that the animal with excellent fattening qualities is characterized by a big chest circumference, a long cylinder-shaped barrel, low height, deep position of chest bone, short lower legs, small lungs, and little respiratory activity.

Apparently Kögel (*8*) was the first to measure the capacity of the chest cavity and the volume of the collapsed and inflated lungs. He showed that the breastbone was longer in proportion to length of chest and lumbar vertebrae, the chest cavity was larger, the lungs were heavier and larger in proportion to live weight, and the heart was relatively heavier in the late-maturing Merino than in the early-maturing Southdown sheep. Röloff (*8*) arrived at similar conclusions, and pointed out in addition that size of chest depends mainly on length of chest and height of chest cavity. Nathusius (*8*) showed that speed performance in horses increased with decreasing breast circumference and decreasing substance. There is uncertainty, in some cases, as to whether the data representing size or weight of lungs and heart are absolute or relative. If they are based on absolute values, consideration of fat deposition in the region of the chest might temper some of the conclusions. Martiny (*1*) in discussing size of lungs, stressed the importance of their quality and suggested that the small lung may be more efficient than the large one—efficiency being dependent on amount of respiratory surface, degree of elasticity, and development of lung capillaries. He reasoned that lung weight increases with the increase of interstitial framework at the expense of both lung tissue and physiological efficiency. The same view was expressed by Nathusius (*1*) and by Lehmann (*1*). Von Lützow (*7*) stated, as reported by Becker (*1*), that the performance of the lung depends first on the volume of the chest and second on the weight of the lung per cubic centimeter of chest volume—the smaller the weight of the lung the better.

In the case of the heart, however, Colin (*8*) concluded that strength is in direct proportion to heart weight. In two horses of different size he found the lighter lungs in the heavier animal; but the heart was definitely heavier in the heavier horse, except on the basis of live weight, where the difference was negligible. Lehmann (*1*) also contended that increased size in the heart (a muscular organ) is associated with increased function. Von Lützow and Müller (*8*) showed that trotting horses had heavier hearts and lungs than walking horses, in relation to chest width, chest circumference, and live weight. Külbs (*8*) found that exercise definitely increased the relative weight of heart and lungs and the circumference of chest in dogs. The organs, however, showed no microscopic or microchemical changes. Kraemer (*8*) showed an increase in chest circumference in cattle when grazing, although the animals actually lost in live weight. Increase in chest circumference was attributed partly to better growth of the inner organs as a result of increased activity and partly to the muscular development in the region of the chest as a result of exercise. In discussing Schneider's work with cattle Mierswa (*8*) made the generalization that weight of the inner organs increases with increasing body weight but usually at a slower rate so that the heavier animals do not show a degree of development equal to that of the lighter ones. He indicates also that females usually have proportionately heavier inner organs than males. Seeberger (*15*), as reported by Mierswa (*8*), is credited with showing that in sheep, chest width is negatively correlated with lung size and weight, but that length and depth of chest are positively correlated with size of lungs. He also showed that the lungs were relatively heavier in young than in old animals and relatively heavier in females than in males, and that both heart and lung weights were relatively greater in the late-maturing fertile Franconia sheep than in the early-maturing, easy-fattening Southdowns. From his anatomical studies of swine Semmler (*8*) found that large circumference and width of chest cavity were associated with smaller lungs, but that the greater lengths of chest cavity were associated with greater heart and lung weight. He also showed that brood sows had larger lung volumes than castrated animals used for fattening. Mierswa (*8*) expressed the belief that heavier lungs indicate superior performance, provided the weight is correctly obtained.

Mierswa's comments regarding the report of Swett, Graves, and Miller (*17*) are worthy of note as they confirmed, according to his interpretation, many of the results reported by others The points he stressed were the greater absolute chest capacity especially the depth and length in the lighter dairy cow, whereas the heavier Aberdeen Angus had a heart of approximately the same absolute (smaller relative) weight, and presumably smaller lungs because the chest cavity was smaller.

Becker (*1*) compared the conformation and anatomy of two breeds of cattle, paying particular attention to the external and internal measurements of the chest and the size of heart and lungs. The Simmentals and Oldenburgs studied were of about the same live weight (average 606 and 596 kg respectively) and measured almost the same in height and in circumference and width of chest. The Oldenburgs, however, were much longer and considerably deeper in the chest and had greater depth of thoracic cavity, but the breeds differed little in width of thoracic cavity. The thoracic index (external depth of chest÷external width of chest), and the heart weight and lung weight—especially the latter—were greater in the Oldenburgs. Moreover, the heart and lung indexes I, II, and III (weight of organ÷circumference, depth, and width, respectively, of thoracic cavity) averaged greater in every case for the Oldenburgs, although the difference was less marked for the heart than for the lungs. The differences varied somewhat at different ages. In relation to both live weight and carcass weight the heart and lungs were larger in the Oldenburgs. In the Simmental breed the higher values were found at early ages, whereas in the Oldenburg breed the values increased with age. The greatest differences therefore occurred with the more advanced ages. These results indicate that the large heart and lungs are found in animals having chests that are deep in proportion to their width.

Mierswa's own studies (*8*) have a very close bearing on the relation of external form to internal anatomy. In comparing milk-, intermediate-, and meat-type breeds of cattle Mierswa found definite associations between external body measurements, internal chest measurements, and the size of the heart and lungs. This was noted particularly in chest lengths and chest depths, while chest width was found to be of little if any significance. In animals with equal chest index (average of the three chest dimensions) and with equal live weight, the thoracic capacity was greater in females than in males. Size of chest cavity was greatest for the dairy-type, less for the intermediate-type, and least for the beef-type breeds. In proportion to live weight the heart was heavier in females than in males, and among the females was heaviest in the milk-type and lightest in the meat-type breeds. No general relations were found in studying the relative lung weight. Mierswa pointed out that all substantial differences shown in the relation between outer and inner chest measurements and between external measurements and heart and lung development, are dependent on the ability of the different breeds and types to put on flesh or fat around the chest. Mierswa's findings were in some respects contrary to those of Korreng (*5*), which indicated that relative heart weight was greater in the male than in the female and lung weight was greater in the female.

Von Lützow (*7*) found that volume of thoracic cavity and lung weight were greater in slow horses than in fast ones, even though the heart weight was proportionately larger in fast horses. Müller (*11*, *12*) disagreed with some of Von Lützow's conclusions, pointed out inaccuracies resulting from estimating volume of thoracic cavity, and stated that lung weight cannot be estimated from its volume on account of variation in its specific gravity. Seeberger (*15*) concluded from his study of sheep that a thoracic cavity with a large index (high width in relation to depth) is correlated with a smaller lung.

A comprehensive study of the effect of age and plane of nutrition on body form, internal anatomy, and chemical composition of the different parts of the body, begun at the Missouri Agricultural Experiment Station in 1906 (*9*), shows the anatomic structure of the animals when slaughtered. The ratio of empty weight to live weight was greatest at birth, least at 8½ months of age, and intermediate at 4 years. Animals on low planes of nutrition had low ratios. The ratio of carcass weight to live weight decreased with age to 8½ months and then increased to a maximum at 3 to 4 years of age. The better-fed animals had the highest ratio. On the basis of empty weight the carcass weight increased continuously with age up to 3 or 4 years. Hide and hair, heart, brain and spinal cord, and intestine length decreased in relation to empty weight with an increase in age and fatness. The weights of blood, lungs, stomachs, intestines, liver, kidneys, spleen, and pancreas, however, increased in relation to empty weight up to about 8½ months, after which the relation showed a decrease. The stomachs and liver showed marked retardation on the low planes of nutrition. The limited number of animals and the fact that they represented different degrees of fatness and were slaughtered at different ages, made any attempt to correlate organ size with body dimensions impracticable. A comparison of the anatomy of cows representing different degrees of fatness, on the basis of percentage of empty body weight (*10*) showed that the fat cows had low values for weights of carcass, heart, lungs, brain and spinal cord, stomachs, intestines, and spleen; high values for offal fat and liver; and approximately the same values for hide, blood, and pancreas. With fattening, most of the internal organs decreased or remained constant in relation to empty body weight.

The Bureau of Dairy Industry (*17*) has shown that, although a highly specialized dairy cow differed greatly from a highly specialized beef cow in external form, the internal organs did not differ sufficiently in size and weight to indicate significant differences in function. The skeletal structure of the cows was generally similar, indicating the great effect of fleshing on external body form. The comparison of a cow and a bull of the same breed (*18*) also showed that the external form of the two animals differed greatly in certain particulars, especially in width and circumference of body and general heaviness of the anterior parts, but that there was little difference in body depth or scale. Only two of the internal organs - the thyroid and the spleen—were larger in relation to empty body weight in the bull than in the cow. The skeletons of the cow and bull were similar except for the head, the cervical vertebrae, and the shape of the pelvis. The differences in body form in this case also were due chiefly to differences in muscular development and fleshing. A study of the comparative conformation, anatomy, and udder characteristics of cows of certain beef and dairy breeds (*19*) showed marked differences in body form. Cows of the Hereford, Holstein-Friesian, and Aberdeen Angus breeds were found to be generally similar in skeletal structure. The most marked differences in external body proportions were associated with measurements most directly affected by fat deposition. There was a tendency for the internal organs to be largest in the Holsteins. In relation to empty body weight, however, in most cases the Herefords had the largest, and the Aberdeen Angus the smallest internal organs. Apparently as fleshing increases the total size or weight of the animal structure, the organs become smaller in relation to empty body weight.

A recent publication[4] having a particularly close bearing on the present work is a progress report on the study of conformation and anatomy of dairy cows in relation to their producing capacity. This work is being conducted by the Bureau of Dairy Industry in cooperation with a number of State agricultural experiment stations. As the data are not yet sufficient in number, correlations were not given and conclusions were not drawn. The report gave the average, maximum, and minimum weights and measurements, together with averages for large, medium, and small cows in five different breed groups. Data were given for about 120 different ante-mortem and post-mortem items, and 593 cows having records of production were included.

Joseph (*4*) found that the ratio showing the number of grams of heart weight per kilogram of live weight varies from 2.36 and 2.76 for the tame and wild rabbit, respectively, to 11.55 for the deer and 25.64 for the thrush. Other ratios are 4.52 for the pig, 3.83 for the cow, 5.85 for the ewe, 5.81 for the horse, and from 7.43 to 9.71 for dogs. There seems to be some relation between physical activity and ratio of heart weight to live weight.

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[4] Swett, W. W., Matthews, C. A., Miller, F. W., and Graves, R. R. VARIATIONS RECORDED IN THE STUDY OF THE CONFORMATION AND ANATOMY OF 593 DAIRY COWS HAVING RECORDS OF PRODUCTION. U. S. Bur. Dairy Indus., BDIM—589, 23 pp. 1937. [Mimeographed.]

Stewart (*16*) studied the relation of organ size to body weight and body-surface area in dogs. He found that the spleen and heart weights were in general proportional to body weight, whereas the liver, stomach, intestine, kidney, and lung weights were more nearly proportional to body-surface area. Roaf (*14*), on the contrary, found that the ratio of heart and liver weights to body weight in cats varied independently of body weight, whereas the ratio of kidney weight to body weight varied directly with body weight.

Kraemer (*6*) studied the skull capacity and brain weight of domestic and wild animals, fishes, and human beings. He found the brain to be generally larger in males than in females and he found also greater absolute brain weights in large than in small animals. As a rule the smaller individuals had the larger brains in relation to their live weight.

EXPERIMENTAL PROCEDURE

METHODS OF OBTAINING ANTE-MORTEM DATA

The usual procedure in the stockyards at the time these data were obtained was to unload the cattle in the morning and to feed and water them immediately. Buyers for the packing company then proceeded to make purchases for the day. From such purchases cows of certain types were selected for this study, tagged for identification, and weighed individually during the late morning or early afternoon of the same day. The cows selected represented a wide range in type or conformation, an effort being made to include not only the deep, wide, well-developed individuals, but also the narrow, shallow animals, which theoretically should be deficient from the standpoint of constitution and capacity. Most of the cows selected showed definite characteristics of some dairy breed.

Usually the cows were held in small stockyard pens during the rest of the day and night, with access to water but not to feed. In most instances they were driven to the larger packing-house pens early the following morning, although a few were moved in the late afternoon of the day of purchase.

During the morning following the day of purchase, the external body measurements (ante-mortem data), consisting of 17 body measurements and a number of observations, were obtained for each animal. The slaughtering of these animals took place on the afternoon of the same day except in a few instances when they were slaughtered just before noon, immediately after the completion of the ante-mortem measuring. Approximately 24 hours elapsed, therefore, between last feeding and the time of measuring; approximately 18 hours between weighing and measuring; and about 24 hours between weighing and slaughtering.

Considerable individual variation was found in the readiness with which the cows permitted handling. Some evidently had come from dairy herds and were accustomed to daily handling. Others apparently had not been handled regularly, and some difficulty was experienced in holding them in the desired position for measuring. In such cases incorrect position of the feet may have slightly affected such measurements as height at withers and some of the dimensions of the chest. However, all the ante-mortem data except those for live weight were obtained by one person and are as uniform and accurate as could reasonably be expected.

Figure 1 illustrates the landmarks, or points of anatomy, at which the different external body measurements were taken.

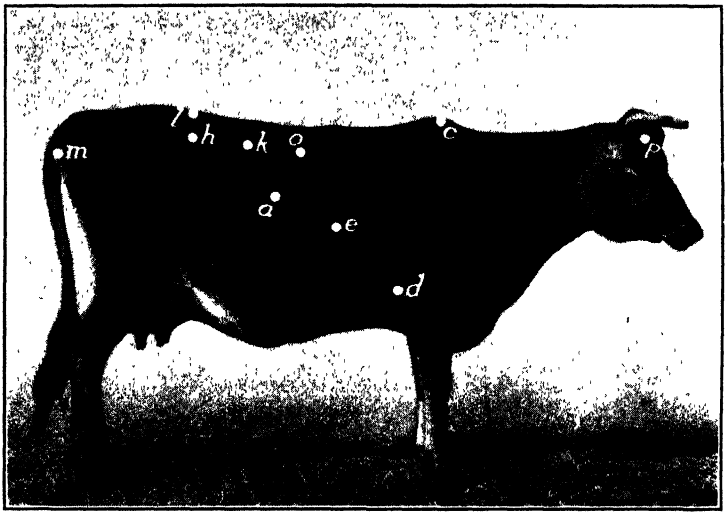

FIGURE 1.—Landmarks, or points of anatomy, used in making the body measurements. See text for explanation of points.

A list of the ante-mortem items recorded, together with details concerning the methods employed in obtaining them, are given below. Body weight is recorded in pounds and body measurements in centimeters. The first few items, which are descriptive rather than measurements of conformation, do not enter into the tabulations.

ANTE-MORTEM ITEMS RECORDED

Identification of animal.
General source (State from which animal was received).
Breed.
General type.
General condition
Handling quality of hide at center of thirteenth rib. Taken at *a* (fig. 1).
Thickness of fold of hide at center of thirteenth rib. Measured with vernier caliper at *a*.
Live weight.
Height at withers. The point represented by *c* is directly above and directly in line with the front leg when squarely placed.
Depth of fore chest. Taken with vertical caliper through the vertical transverse plane passing just to the rear of the point of the elbow (ulna), at *d*.
Depth of rear chest. Taken with vertical caliper through the vertical transverse plane passing through the center of the tenth rib (the fourth rib from the rear), at *e*.
Depth of paunch. Taken with vertical caliper through the vertical transverse plane passing through the center of the thirteenth (or last) rib at *a*.
Width of fore chest. Taken with horizontalcaliper through the vertical transverse plane passing through *d*.
Width of rear chest. Taken with horizontal caliper through the vertical transverse plane passing through *e*.

Width of paunch. Taken with horizontal caliper through the vertical transverse plane passing through *a*.

Width of hips. Taken with horizontal caliper touching lateral points of hips (tuber coxae) at *h*.

Width of loin. Taken with horizontal caliper touching lateral edges of center of loin at *k*.

Length of loin. Taken with tapeline from the lateral face of hip point (tuber coxae) forward to the highest point along the posterior edge of the thirteenth rib to which a finger or thumb can be forced (*h* to *o*).

Length, withers to hips. Taken with a tapeline from withers to a median point on the spine midway between tops of hip points (*c* to *l*).

Length, hips to pinbones. Taken with tapeline from a point on spine midway between tops of hip points to posterior tip of pinbone (from *l* to a point near to and directly above *m*).

Length, withers to pinbones. Sum of lengths from withers to hips and from hips to pinbones.

Circumference of fore chest. Taken with tapeline through the vertical transverse plane passing through *d*.

Circumference of rear chest. Taken with tapeline through the vertical transverse plane passing through *e*.

Circumference of paunch. Taken with tapeline through the vertical transverse plane passing through *a*.

Width of head. Taken with tapeline between extreme lateral edges of forehead at narrowest point (*p*), midway between eyes and the poll. (In measuring width of head, since the edges of the skull are somewhat posterior to the frontal face of the forehead, the tape curves over the face and registers a value slightly in excess of the true width at *p*.)

METHODS OF OBTAINING POST-MORTEM DATA

The cows selected for the day's test were slaughtered at the end of the day's killing, after the slaughterhouse routine had been stopped for a brief period. The rapidity of killing was somewhat reduced for the test group, but otherwise the usual procedure was followed, except for slight variations necessitated by the weighing and measuring of the organs and body parts. Before the organs and parts of an animal's body were separated for distribution to the different divisions of the slaughterhouse, each was given an identification mark corresponding to one on a tag affixed to the ear. Nearly all organs of the body were weighed, and some were measured, as they passed through the different divisions of the packing house.

The work of collecting data required the services of from 15 to 20 men of the Bureau of Animal Industry, in addition to the regular employees of the packing house, for a part of each day over a period of nearly a month. Selected employees were stationed in groups at each of the places where data were to be obtained. Each group was equipped with special scales and other equipment used in obtaining the data. One member of the group was assigned to record the data on each of six or more groups of organs, as the other members prepared them. In this manner the data were collected almost simultaneously and in a brief period of time. In most cases identification of the different organs and parts of each animal was accomplished readily as they reached the different processing points in the slaughterhouse. However, some items for some animals had to be eliminated because of pathological conditions or because of failure to identify the organ or part with any particular individual. Thirty-one post-mortem items were recorded in addition to several internal measurements of the thoracic cavity. Complete post-mortem data were obtained on more than 200 cows and partial data on more than 350 cows.

The nature of the organ or part and the relative difficulty involved in its preparation may have affected the results slightly. Some organs were more easily and uniformly weighed or measured than others. For example, in some cases a small quantity of blood was lost; the pancreas, an irregularly shaped organ, was subject to some variation in preparation; and there was some lack of uniformity in separating the brain from the spinal cord. The weight of the pituitary body is omitted from all tabulations because the scale used did not permit weighing in sufficiently fine units, and because in its preparation an excessive quantity of membrane was left attached in some cases. In the usual procedure of handling the stomachs in the packing house, the omasum was "hashed" with its contents and made into tankage. In order to determine the weight of the omasum, 50 omasa were hashed but recovered and washed for weighing, the average weight for the 50 being added to the total for the other three divisions of the stomach for each cow to make up the total empty weight of stomach. A slight error is introduced in obtaining the total stomach weight in this manner. Moreover, the necessity for using six or more groups of individuals, in taking and recording the post-mortem data, and the fact that the personnel of some of these groups changed somewhat from day to day, may have introduced slight variations in methods, but the data are believed to be satisfactorily uniform and accurate.

The following is a descriptive list of the items for which post-mortem data were recorded, although not all of them are included in the tabulations that appear later in this paper.

POST-MORTEM ITEMS RECORDED

Identification of animal.
Live weight (pounds).
Weight of blood (pounds).
Weight of hide (pounds).
Weight of caul fat (pounds).
Weight of fetus, if cow was pregnant (pounds).
Weight of lungs (after separation from trachea) (pounds). Taken for (1) right lung, (2) left lung, and (3) total, both lungs.
Weight of heart after removal of fat (pounds).
Circumference of heart after removal of fat (centimeters). Taken (1) in a plane parallel to and close to base, and (2) over the apex in a plane passing through the apex.
Weight of thoracic fat (pounds).
Weight of liver after removal of gall bladder and fat (pounds).
Weight of intestines and contents. Taken as removed, with fat and mesentery attached (pounds).
Weight of intestine fat and mesentery (pounds).
Weight of empty intestines (pounds). Taken for (1) small intestine, (2) large intestine, and (3) total of both.
Weight of contents of intestines (pounds). Represents difference between weight of intestines with contents and weight of empty intestines and fat.
Length of intestines (feet). Taken for (1) small intestine, (2) large intestine, and (3) total of both.
Weight of stomach and contents with fat removed (pounds).
Weight of stomach fat (pounds).
Weight of stomach after being emptied and washed (pounds). Taken for (1) rumen, reticulum, and omasum;[5] (2) abomasum, and (3) total for all four divisions.
Weight of contents of stomach (pounds) (calculated by difference).
Weight of total abdominal fat (pounds).

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[5] Weight of omasum not determined for each cow. Average of 50, cleaned and weighed together, used in all cases

Weight of pancreas[6] (ounces).
Weight of spleen (pounds).
Weight of brain (ounces).
Weight of pituitary body[7] (ounces).
Measurements of thoracic cavity of dressed carcass, as follows:

(1) Maximum dorsoventral diameter of thoracic cavity (centimeters). Taken from spinal cord at each thoracic vertebra to sternum or to diaphragm.
(2) Maximum length of thoracic cavity (centimeters). Taken with tapeline from the extreme posterior point, at attachment of diaphragm, to the anterior edge of the first rib.
(3) Maximum internal lateral width of thoracic cavity (centimeters). A measure of the concavity of the split thorax, taken at the seventh rib.

Weight of carcass (pounds).

SUPPLEMENTAL VALUES CALCULATED FROM ANTE-MORTEM AND POST-MORTEM DATA

A number of supplemental values have been calculated from the original ante-mortem and post-mortem data to facilitate the study of comparative conformation and anatomy. Many of the supplemental values represent body proportions in the form of ratios or percentages. Those which represent body-surface area; volume of barrel; cross-section areas of fore chest, rear chest, and paunch; and volume of thoracic cavity are considered to be close approximations and reasonably significant, but no claim is made as to their exactness. Following is a discussion of the derivation of the supplemental data.

(1) The body-surface area is supposed to be closely related to metabolism. For this reason it was calculated for all animals by means of the formula of Hogan and Skouby (*3*): $S=W^{0.4}\times L^{0.6}\times K$, in which S=surface area in square centimeters, W=live weight in kilograms, L=length of body in centimeters, and K=217, a constant value for cattle. The live weight as taken in pounds has been converted to kilograms. The length from the withers to a line between the hips added to the length from a line between the hips to the pinbones, gives the total length of the body (withers to pinbones).

(2) Legginess is a term frequently used to denote the proportion of the animal's height located between the ventral surface of the torso and the ground, or the proportion of the length of the legs to the total standing height of the animal. In this study legginess is determined by subtracting the depth of fore chest from the height at withers and dividing the difference by the height at withers.

(3) Cross-section area of fore chest, a term applied to the area of an ellipse having as its axes the two maximum diameters of the fore chest, is used to represent the cross-section area of the body through the fore chest. This value is derived by multiplying the depth of fore chest by the width of fore chest, and the product by 0.7854. This area is undoubtedly somewhat in excess of the actual cross-section area of the fore chest, because depressions occur in many cows, posterior to the scapula and ulna.

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[6] Dissection not perfectly performed. Accuracy of weights subject to some question

[7] Scales used were not sufficiently delicate for weighing so small an organ. Completeness of dissection subject to question

(4) The cross-section area of rear chest is the product of the depth of rear chest, the width of rear chest, and the factor 0.7854. This appears to give a close approximation of the cross-section area through this plane as the contour of the body is usually reasonably regular at that point.

(5) The cross-section area of paunch is the product of the depth of paunch, the width of paunch, and the factor 0.7854. A close approximation of the cross-section area at that point also should be obtainable in this manner because of the fairly regular contour lines at that plane in most cows.

(6) The volume of barrel, which is generally associated with capacity for feed consumption and producing ability in the cow, is calculated by multiplying the average of the cross-section areas of the fore chest and paunch by the linear distance from the withers to a median point on the spine midway between the tops of the hip points.

(7) Thoracic index, a term used in referring to the "build" of humans, is the ratio of the depth to width of chest and is determined for cattle by dividing the depth of fore chest by the width of fore chest.

(8) The rear-chest index is the ratio of the depth of rear chest to width of rear chest.

(9) The abdominal index, which is the ratio of depth to width of paunch, is determined by dividing depth of paunch by width of paunch.

Obviously a high index for fore chest (7), rear chest (8), or paunch (9) indicates that the animal was deep in relation to width of body at the point measured. The indexes may be used, therefore, as a basis for classifying animals, with regard to the proportions of the trunk.

(10) Wedge shape, which refers to the angular form of the body, is generally considered desirable in dairy cows. For comparative purposes it can be expressed in terms of the relation of the fore chest to the paunch dimension in depth, width, or circumference. This relation, for each of these dimensions, is shown as a simple ratio of paunch measurement to fore-chest measurement obtained by dividing the former by the latter. It is also shown in actual units of measurement as the difference between the chest measurements and the corresponding paunch measurements.

(11) Volume of thoracic cavity is difficult to calculate because of its irregular shape and outline and the flexibility of the diaphragm, and at best it can only be approximated. At the same time some measure of the thoracic capacity is desirable as it represents the space available for the heart and lungs. An attempt has been made to calculate the volume or capacity of the thoracic cavity on the basis of two frustums of cones having a common base. It is apparent that these figures cannot be truly conical in shape because their bases are elliptical rather than circular. The formula for determining the area of an ellipse was used in calculating the base areas, and the formula for calculating the volume of a frustum of a cone was then applied.

The size of the forward or anterior frustum is found as follows: Complete measurement of the skeleton of a dairy cow shows that the axes of the thoracic cavity at the extreme anterior point (first rib) are approximately in the proportion of 2 to 1. The area of the anterior base A of the anterior frustum is approximated when the depth of cavity at the first thoracic vertebra is multiplied by one-half itself, and this product is multiplied by 0.7854. The posterior base C of the anterior frustum, which is the common base of both frustums, has as axes the maximum interior depth and the interior lateral width of the thoracic cavity at or near the seventh rib. The maximum interior depth does not necessarily come at exactly the seventh-rib plane, but is not far distant. The area of the common base C therefore is the product of these two axes times 0.7854. The altitude of the anterior frustum is assumed to be one-half the maximum length of the thoracic cavity, as measured from the anterior edge of the first rib to the posterior point of attachment of the diaphragm. The volume of the anterior frustum is:

$$(\text{Base } A+\text{base } C)+\sqrt{\text{base } A\times\text{base } C}\times\frac{\text{length of thoracic cavity}}{6}.$$

The volume of the posterior frustum is determined similarly. In this frustum base C, which is common to both frustums, represents its anterior base. Its posterior base, P, which is indefinite in shape and outline, is assumed to have axes in the proportion of 2 to 1, with the lateral axis twice the vertical one. The vertical axis is measured as the dorsoventral diameter of the cavity at the most posterior vertebra in the cavity. To obtain the area of base P this last dorsoventral, or vertical, dimension is multiplied by twice its value, and the product by 0.7854. The volume of the posterior frustum is:

$$(\text{Base } C+\text{base } P)+\sqrt{\text{base } C\times\text{base } P}\times\frac{\text{length of thoracic cavity}}{6}.$$

The volume of the entire thoracic cavity is the sum of the volume of the anterior and posterior frustums.

(12) Percentage of heart weight to live weight is determined by dividing the weight of heart by the live weight.

(13) Percentage of lung weight to live weight is determined by dividing the total weight of lungs by the live weight.

PRESENTATION OF DATA

VARIATION

The variation in the magnitude of the range in the different measurements was marked, but the variation in the post-mortem data appeared to be much greater than in the ante-mortem data. The results of an analysis of the degree of variation in the ante-mortem and in the post-mortem data are given in tables 1 and 2.

Tables 1 and 2 enumerate the items studied and show the units in which the measurements were recorded. Averages of the lowest five and the highest five values instead of the lowest and highest single values were used to avoid attaching undue importance to individual extremes in showing the range of the data obtained. One column gives the arithmetic average of the entire lot of measurements for each item. Another column shows the median or midmost measurement in the entire population. If there were 361 measurements for a given item, the median would be the one hundred and eighty-first value from both the highest and the lowest. In the case of 362 animals in the group, the median would be halfway between the one hundred and eighty-first and the one hundred and eighty-second values, counted from either the highest or the lowest. The standard deviation with its probable error is given to show the actual dispersion of the individual measurements for each item, and the coefficient of variation with its probable error is given to show the comparative measure of dispersion for different items.

TABLE 1.—*Variations in the ante-mortem data obtained from cows slaughtered in a packing house*

Item [1]	Minimum measurements (average of 5 lowest)	Maximum measurements (average of 5 highest)	Arithmetic average (of all measurements)	Median (the midmost measurement)	Standard deviation and probable error	Coefficient of variation and probable error
Data as obtained						
Thickness of hide (361) [1] centimeters	0.51	1.15	0.78	0.77	0.12±0.00	15.86±0.40
Live weight (362) pounds	738	1,424	1,059.67	1,070.00	132.59±3.32	12.51± .31
Height at withers (362) centimeters	117.30	141.20	129.02	129.00	4.82± .12	3.73± .09
Depth of fore chest (362) do	65.50	81.40	72.61	72.50	2.99± .07	4.11± .10
Depth of rear chest (362) do	64.40	80.80	72.35	71.50	3.30± .08	4.56± .11
Depth of paunch (362) do	62.30	82.90	72.83	73.00	4.01± .10	5.50± .14
Width of fore chest (361) do	33.70	50.90	41.47	41.50	3.35± .08	8.08± .20
Width of rear chest (362) do	45.60	65.00	55.93	56.00	3.91± .10	7.00± .18
Width of paunch (362) do	45.50	70.00	58.02	58.00	4.82± .12	8.31± .21
Width of hips (362) do	45.50	61.90	53.81	54.00	2.96± .07	5.50± .14
Width of loin (361) do	29.20	41.70	35.78	36.00	2.33± .06	6.52± .16
Length of loin (334) do	26.00	45.00	34.29	34.00	3.25± .08	9.47± .25
Length, withers to hips (362) do	81.20	105.20	93.51	94.00	4.54± .11	4.85± .12
Length, hips to pinbones (361) do	45.20	58.20	50.80	51.00	2.53± .06	4.97± .12
Length, withers to pinbones (361) centimeters	129.80	160.60	144.31	144.00	5.98± .15	4.15± .10
Circumference of fore chest (362) centimeters	165.80	210.20	186.41	186.00	8.37± .21	4.40± .11
Circumference of rear chest (361) centimeters	180.60	233.20	206.90	207.00	10.72± .27	5.18± .13
Circumference of paunch (362) do	177.80	242.20	211.56	211.00	12.38± .31	5.85± .15
Width of head (362) do	19.40	26.60	22.91	23.00	1.56± .04	6.82± .17
Calculated values showing body bulk						
Body-surface area (361) square centimeters	41,666	59,909	50,615	50,674	3,454±87.00	6.82± .17
Volume of barrel (361) cubic centimeters	185,853	357,599	266,990	267,546	34,714± 871	13.00± .33
Cross-section area of fore chest (361) square centimeters	1,823.62	3,124.91	2,368.24	2,353.84	247.62± 6.22	10.46± .26
Cross-section area of rear chest (362) square centimeters	2,395.63	3,990.38	3,182.50	3,192.65	330.40± 8.28	10.38± .26
Cross-section area of paunch (362) square centimeters	2,298.71	4,300.46	3,327.22	3,326.17	404.13±10.13	12.15± .30
Calculated values showing body proportions:						
Legginess (362)	0.378	0.485	0.437	0.438	0.02± 0.00	4.08±0.10
Thoracic index (361)	1.49	2.07	1.76	1.74	.13± .00	7.42± .19
Rear-chest index (362)	1.135	1.508	1.298	1.295	.07± .00	5.73± .14
Abdominal index (362)	1.067	1.499	1.261	1.259	.09± .00	6.98± .17
Wedge shape: [2]						
Depth, ratio (362)	.878	1.137	1.004	1.007	.04± .00	4.29± .11
Width, ratio (361)	1.170	1.661	1.403	1.402	.10± .00	7.21± .18
Circumference, ratio (362)	1.032	1.254	1.134	1.134	.04± .00	3.93± .10
Depth, units (362)	−9.20	+8.20	+.22	+.50	3.03± .08	13.65± .34
Width, units (361)	7.50	26.60	16.59	16.50	3.80± .10	22.90± .57
Circumference, units (362)	5.80	46.80	25.08	25.00	8.26± .21	32.93± .83

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[1] Numbers in parentheses show number of animals represented.

[2] For explanation of items see p. 209.

TABLE 2.—*Variations in the post-mortem data obtained from cows slaughtered in a packing house*

Item [1]	Minimum measurements (average of 5 lowest)	Maximum measurements (average of 5 highest)	Arithmetic average (of all measurements)	Median (the midmost measurement)	Standard deviation and probable error	Coefficient of variation and probable error
Weight of blood (362) pounds	25.05	65.40	40.69	40.00	7.33±0.18	18.02±0.45
Weight of hide (353) do	47.80	86.60	64.00	63.00	7.60±.19	11.87±.30
Weight of lungs (234) do	4.66	11.57	7.36	7.18	1.28±.04	17.40±.54
Weight of heart (260) do	3.09	5.80	4.42	4.44	.59±.02	13.27±.39
Circumference of heart at base (237) centimeters	34.40	53.68	44.12	44.00	3.58±.11	8.11±.25
Circumference of heart over apex (237) centimeters	41.40	61.00	50.42	50.00	3.49±.11	6.93±.21
Weight of liver (244) pounds	8.52	18.20	12.91	13.00	1.85±.06	14.30±.44
Weight of empty intestines (277) do	16.22	29.71	21.36	21.12	2.60±.07	12.15±.35
Length of intestines (297) feet	106.00	180.50	139.46	137.00	13.45±.37	9.64±.27
Weight of empty stomach (311) pounds	26.90	52.60	38.84	38.60	4.81±.13	12.40±.34
Weight of pancreas (268) ounces	1.48	16.75	7.46	7.06	3.26±.10	43.76±1.27
Weight of spleen (245) pounds	1.16	2.69	1.77	1.75	.31±.01	17.54±.53
Weight of brain (321) ounces	10.40	19.18	15.60	15.62	1.51±.04	9.66±.26
Weight of thoracic fat (230) pounds	1.53	7.20	3.25	2.97	1.18±.37	36.39±1.14
Weight of abdominal fat (316) do	8.90	64.90	25.49	23.00	10.95±.29	42.97±1.15
Depth (dorsoventral) of thoracic cavity (274) centimeters	43.80	56.20	49.59	49.00	2.23±.06	4.50±.13
Length (anterior-posterior) of thoracic cavity (274) centimeters	57.00	80.20	70.69	71.00	4.12±.12	5.83±.17
Width (lateral) of thoracic cavity (270) centimeters	30.30	41.60	35.59	36.00	2.35±.07	6.60±.19
Weight of carcass (358) pounds	340.20	789.00	537.64	537.00	84.87±2.14	15.78±.40
Volume of thoracic cavity (267) cubic centimeters	53,574	102,860	75,087	74,749	10,092.29±294.60	13.44±.39
Heart weight per 100 pounds of live weight (260) pounds	0.28	0.55	0.41	0.41	.05±0.00	12.15±.36
Lung weight per 100 pounds of live weight (234) pounds	.45	1.06	.69	.67	.12±.00	17.26±.54

[1] Numbers in parentheses show number of animals represented.

VARIATION IN ANTE-MORTEM DATA

Nineteen of the items in table 1 represent the data in units of weight or measurement as obtained on the living animal. Of these 19 items 17 are primarily measurements of skeletal size, 1 (live weight) is a measure of mass, and 1 (thickness of hide) presumably has no relation to either skeletal size or mass. In addition, there are 5 calculated items designed to show a measure of body bulk and 10 calculated items which show body proportions.

The arithmetic average and the median are almost identical for each item in the ante-mortem data, indicating a normal distribution which centers near the average.

The standard deviation, of course, tends to increase in magnitude with an increase in the magnitude of the measurement it represents. The significance of the standard deviation for each item in relation to the arithmetic average for that item, is shown by the coefficients of variation which are given in table 1, and discussed in the following paragraphs.

In the group of 19 items of weights and measurements, thickness of hide and live weight have the highest coefficients of variation. Length of loin, with a coefficient of 9.47, has the next highest, followed by the five measurements for width of body. The items in this group showing the smallest coefficients of variation are the height at withers, 3.73, depth of fore chest, 4.11, and length from withers to pinbones, 4.15. In general, the items in this group that indicated depths, with an average of 4.72 for the coefficients, lengths, with an average of 5.86, and circumferences, with an average of 5.17, were less variable than the items that indicated width of body, for which the coefficients averaged 7.04, with the single exception of the length of loin.

The coefficients of variation for the calculations showing body bulk or size, and those for the calculations showing body proportions, were greater on an average than those for items indicating skeletal size. The average of the coefficients for each group was 10.56 for the calculations showing body bulk, 10.91 for the calculations showing body proportions, and 5.83 for the measured items showing skeletal size. The greatest variations were found in connection with the measurements affected by condition of flesh and "fill" of the animal; and the least in connection with body height and the body lengths that represent body scale.

VARIATION IN POST-MORTEM DATA

Variations have been studied for 22 post-mortem items. These include 19 items based on weights or measurements as taken, 2 ratios that show the size of heart and lungs in relation to live weight, and 1 calculated value giving an approximate measure of the volume of the thoracic cavity. Although the two lobes of the lungs, the large and small intestines, and the different divisions of the stomachs were weighed or measured separately, only the totals for each of these organs are given in table 2.

As in the case of the ante-mortem data, the arithmetic average and the median are almost equal for post-mortem items, indicating a normal distribution centering on the mean.

The three highest coefficients of variation are those for weights of pancreas, abdominal fat, and thoracic fat, and the three lowest are those for the depth, length, and width measurements of the thoracic cavity. Just why the variation in the weight of pancreas was so great cannot be explained. It was probably due in part to differences in trimming, but data secured in another project indicate that there is a great variation in the weight of this organ. The next group in the order of magnitude of their coefficients of variation includes the weights of blood, spleen, lungs, lungs per 100 pounds of live weight, and carcass, with coefficients ranging from 18.02 to 15.78. This group is followed by the weight of liver, volume of thoracic cavity, weights of heart, empty stomachs, empty intestines, heart per 100 pounds live weight, and hide, with coefficients ranging from 14.3 to 11.87. The variations are greater in the weights of the empty intestines and the heart than in the measurements of those organs. The coefficients of variation for the heart and lungs are also much larger than those for the width, length, and depth of thoracic cavity. The coefficients for these last three measurements are quite similar to those for the ante-mortem measurements for depth, width, and circumference of chest. The coefficient of variation for the weight of brain is greater than that for width of head in the ante-mortem data, and the same is true of the coefficients for other measurements of organs as compared with those for skeletal size. For all of the 19 post-mortem items of weight and measurement, the average of the coefficients of variation was 16.16, as compared with an average of 5.83 for the 17 ante-mortem measurements of skeletal size.

In a previous publication (*19*) the post-mortem data were divided into seven groups on the basis of the functions of the organs and body parts, to determine the extent to which those functions are associated with anatomical differences. The items that were included in this study were grouped according to the same plan, as follows: (1) Body-size group, which includes live weight,[8] weights of hide and dressed carcass, and measurements of carcass; (2) nervous-system group, which includes weight of brain; (3) circulation and respiration group, which includes weights of blood, lungs, and heart, and measurements of the heart; (4) digestion group, which includes weights of liver, pancreas, intestines, and stomach, and intestine measurements; (5) urogenital group (not included); (6) endocrine-gland group, which includes weight of spleen; and (7) visceral-fats group, which includes weights of abdominal and thoracic fats. The highest variations occurred in the visceral-fats group, the second highest in the digestion group, and the lowest in the nervous-system (brain) and body-size groups.

RELATION BETWEEN EXTERNAL CONFORMATION AND INTERNAL ANATOMY, AND INTERRELATIONS BETWEEN ANTE-MORTEM AND BETWEEN POST-MORTEM ITEMS

Owing to the large number of variable items under consideration (34 ante mortem and 22 post mortem), the determination of interrelations for all possible combinations of the separate items would be virtually an endless task. However, it appeared practicable to study the relation of the weight or measurement of certain vitally important organs representing different functions and anatomical locations, to the external body measurements (ante-mortem data) and to the size of the other internal organs and body parts (post-mortem data); to determine what relation the live weight of the animal bears to the different measurements of conformation and to the weight and measurement of the internal organs and body parts; and to study the interrelation between some of the external body measurements and between some of the different items of internal anatomy.

In addition to live weight, the weight of heart, the weight of lungs, and the length of intestines were chosen for correlation studies. The heart and lungs were chosen because they have long been associated with theories concerning the vitality and performing ability of animals, and also because their weights can be determined readily and definitely. The length of intestines was chosen because it can be measured with a high degree of accuracy and it seemed desirable to include in the study a representative part of the digestive system. It was important to include live weight as one of the items of study in order to determine the extent to which the size of the different body parts may be related to, or independent of, the size of the animal.

More than 40 correlation coefficients were determined for each of these four items (heart weight, lung weight, intestine length, and live weight). The results are given in four tabulations, and the correlation coefficients in each tabulation are arranged in the order of descending magnitude. A coefficient of 0.500 or more is generally considered high; one of 0.300 is moderate, and one which does not differ from zero by four times its probable error is not considered statistically significant. The relative significance of the coefficients in each of the lists can be evaluated on this basis. For convenience each list is divided into sections, consisting of coefficients below 0.300 and multiples of 0.100 above that level.

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[8] Since only one live weight is available for each cow used in this study, it is considered with the ante mortem items.

The coefficients of correlation obtained for heart weight in relation to other items are as follows:

	Heart-weight-coefficient
Range, over 0.500:	
Circumference of heart (over apex)	+0.538±0.031
Live weight	+.509± .031
Body-surface area	+.503± .031
Range, 0.400-0.499:	
Circumference of fore chest	+.494± .032
Circumference of rear chest	+.484± .032
Volume of barrel	+.471± .033
Weight of carcass	+.463± .033
Depth of rear chest	+.457± .033
Weight of hide	+.447± .034
Weight of liver	+.440± .035
Circumference of paunch	+.437± .034
Depth of fore chest	+.436± .034
Cross-section area of rear chest	+.431± .034
Depth (dorsoventral) of thoracic cavity	+.427± .036
Cross-section area of paunch	+.424± .034
Cross-section area of fore chest	+.422± .034
Circumference of heart at base	+.416± .036
Weight of spleen	+.415± .037
Length (anterior-posterior) of thoracic cavity	+.410± .037
Volume of thoracic cavity	+.400± .037
Range, 0.300–0.399:	
Weight of lungs	+.394± .038
Depth of paunch	+.392± .035
Height at withers	+.364± .036
Length of intestines	+.353± .037
Weight of blood	+.348± .037
Width of rear chest	+.348± .037
Length, withers to pinbones	+.341± .037
Width of paunch	+.324± .038
Width (lateral) of thoracic cavity	+.321± .040
Width of fore chest	+.315± .038
Range, 0.299 or less:	
Length, withers to hips	+.263± .039
Weight of pancreas	+.158± .043
Weight of empty stomach	+.140± .042
Weight of brain	+.124± .043
Thoracic index	−.113± .041
Wedge shape, depth ratio [1]	+.101± .041
Legginess	−.094± .042
Wedge shape, circumference ratio [1]	+.088± .042
Abdominal index	+.074± .042
Rear-chest index	−.058± .042
Wedge shape, width ratio [1]	+.022± .042

[1] For explanation of item, see p. 209.

Only three of the items in this list have coefficients above 0.500 and may be considered as highly correlated with weight of heart, but a number of items have coefficients closely approaching that level. Conspicuous among the highest coefficients are items that might be considered as very closely associated with the general bulk of the animal body, such as live weight, body-surface area, volume of barrel, weight of carcass, and weight of hide. It is noteworthy that the three body circumferences and the three calculated cross-section areas—also somewhat closely associated with body bulk—all have coefficients above 0.400. Depths of chest and all measurements of the thoracic cavity except width have coefficients ranging between 0.400 and 0.499, showing a fairly significant correlation between the size of the heart and the cavity in which it is located.

The comparatively low coefficients ranging between 0.300 and 0.399 include all items of external and thoracic width; also height at withers and length from withers to pinbones, which are measurements of body scale. One other measurement of body length (withers to hips) has a coefficient below 0.300. The seven ratios and indexes that were calculated to show body proportion are all at the bottom of the list. Among the weights or measurements of the other internal organs in relation to the heart, weight of liver has the highest coefficient (0.440), and the rest have coefficients ranging down to that for weight of brain, which is only 0.124.

In general the weight of heart is rather highly correlated with live weight and the measurements associated with body bulk. It is moderately correlated with depth of chest and depth, length, and volume of the thoracic cavity, but only slightly correlated with body width and width of thoracic cavity, or with height and length of body which represent body scale. It is only moderately correlated with the weight or measurement of the other internal organs, except weight of liver, and for several of them (weights of pancreas, stomachs, and brain) the correlations are not significant. Moreover, it is not significantly correlated with any of the ratios or indexes calculated to represent body proportions.

The list of correlation coefficients obtained for weight of lungs in relation to other individual items is as follows:

	Lung-weight coefficient
Range, over 0.400:	
Volume of barrel	+0.444±0.035
Length, withers to hips	+.437±.036
Depth of fore chest	+.432±.036
Length, withers to pinbones	+.423±.036
Body-surface area	+.418±.036
Depth of rear chest	+.418±.036
Volume of thoracic cavity	+.404±.039
Length (anterior-posterior) of thoracic cavity	+.400±.039
Range, 0.300–0.399:	
Weight of heart	+.394±.038
Depth (dorsoventral) of thoracic cavity	+.383±.040
Depth of paunch	+.379±.038
Circumference of fore chest	+.372±.038
Live weight	+.343±.039
Weight of liver	+.340±.040
Weight of hide	+.340±.039
Circumference of rear chest	+.335±.039
Cross-section area of rear chest	+.333±.039
Cross-section area of fore chest	+.321±.040
Cross-section area of paunch	+.313±.040
Circumference of paunch	+.307±.040
Height at withers	+.303±.040
Width (lateral) of thoracic cavity	+.302±.042
Length of intestines	+.300±.040
Range, under 0.299:	
Weight of carcass	+.295±.040
Weight of spleen	+.295±.042
Weight of blood	+.264±.041

Range, under 0.299—Continued.	*Lung-weight coefficient*
Weight of empty stomach	+0.252±0.041
Width of paunch	+.214± .042
Width of rear chest	+.211± .042
Width of fore chest	+.200± .042
Circumference of heart over apex	+.188± .045
Circumference of heart at base	+.180± .045
Weight of brain	+.160± .044
Legginess	−.129± .043
Rear-chest index	+.090± .044
Weight of pancreas	+.057± .045
Wedge shape, depth ratio [1]	+.043± .044
Wedge shape, circumference ratio [1]	+.031± .044
Abdominal index	+.027± .044
Thoracic index	+.011± .044
Wedge shape, width ratio [1]	+.002± .044

[1] For explanation of item, see p. 209

In most cases the lung-weight coefficients are considerably lower than those for heart weight, the highest coefficient for lung weight being 0.444, as compared with a maximum of 0.538 for heart weight. The lung-weight coefficients, like those for heart weight, are comparatively high for volume of barrel and body-surface area but the other items discussed in connection with the heart-weight list as being associated with body bulk (live weight, weight of carcass, weight of hide, body circumferences, and cross-section areas) all have coefficients below 0.400, including many only slightly above 0.300 and one (for weight of carcass) only 0.295. The coefficients for volume and length of thoracic cavity are above 0.400, and that for depth of thoracic cavity is 0.383, showing a fairly significant correlation between size of lungs and size of the cavity in which they are located.

For some unexplained reason, the coefficients for body lengths in relation to lung weight are near the top of this list and are much greater in magnitude than those obtained in relation to heart weight (0.437 and 0.423 for lung weight as compared with 0.263 and 0.341 for heart weight). Despite this fact, height at withers—a measurement previously associated with body length as representing body scale—has a coefficient of only 0.303 for weight of lungs, which is even lower than that for weight of heart. Coefficients of correlation for body depths with lung weight, though slightly lower in magnitude than with heart weight, are somewhat higher in this list. Here, again, the coefficients for depth of body and for depth of thoracic cavity are definitely greater in magnitude than those for width of body. The seven ratios and indexes showing body proportions are all located in the eight lowest positions and none has a coefficient showing any significance.

Among the coefficients based on the lung weight in relation to other internal organs, that for weight of liver ranks higher than those for the weights or measurements of the internal organs listed except the heart, but neither the liver nor the heart has more than a moderately significant correlation with lung weight. Length of intestines (0.300) has the next highest coefficient of the internal-organ items, the rest having coefficients either of doubtful significance or of no significance.

In general, there is some indication of a moderate correlation between lung weight and certain measurements of body surface, body volume, and measurements of the thoracic cavity. The reason for the lung weight having a much lower coefficient than the heart weight (0.343 and 0.509, respectively) when correlated with live weight is not easily explained, except on the basis that the heart might be expected to have a weight more nearly in proportion to the mass of the body because it is a muscular organ. Coefficients for depth—both external and internal—are definitely higher than the corresponding ones for width. Body proportions as shown by indexes and ratios have no relation to size of lungs. Among the internal organs only the weight of heart—its companion in the thoracic cavity—and the weight of liver and length of intestines are even moderately correlated with weight of lungs. Taken as a whole, the weight of lungs cannot be considered as highly correlated with any of the ante-mortem or post-mortem items studied though some moderately significant coefficients are shown.

The list of correlation coefficients obtained for length of intestines in relation to other items is as follows:

	Intestine-length coefficient
Range, 0.300 and over:	
Weight of empty intestines	+0.387±0.035
Width (lateral) of thoracic cavity	+.353±.037
Weight of heart	+.353±.037
Volume of thoracic cavity	+.337±.038
Length (anterior-posterior) of thoracic cavity	+.301±.038
Weight of lungs	+.300±.040
Range, under 0.300:	
Depth of rear chest	+.287±.036
Live weight	+.268±.036
Weight of liver	+.255±.041
Weight of spleen	+.255±.041
Cross section area of rear chest	+.254±.037
Depth of fore chest	+.252±.037
Body-surface area	+.252±.037
Circumference of fore chest	+.251±.037
Depth (dorsoventral) of thoracic cavity	+.250±.039
Circumference of rear chest	+.250±.037
Weight of carcass	+.244±.037
Weight of hide	+.243±.037
Width of loin	+.240±.037
Depth of paunch	+.236±.037
Circumference of paunch	+.231±.037
Cross-section area of paunch	+.228±.037
Width of hips	+.227±.037
Weight of pancreas	+.218±.040
Cross-section area of fore chest	+.213±.037
Volume of barrel	+.202±.038
Height at withers	+.200±.038
Length, withers to pinbones	+.199±.038
Length, withers to hips	+.192±.038
Width of rear chest	+.176±.038
Width of paunch	+.173±.038
Length of loin	+.168±.038
Weight of empty stomach	+.142±.039
Width of fore chest	+.122±.039
Weight of blood	+.113±.039
Legginess	−.071±.039
Wedge shape, width ratio [1]	+.068±.039
Weight of brain	+.061±.040
Wedge shape, depth ratio [1]	+.057±.039
Wedge shape, circumference ratio [1]	+.053±.039
Thoracic index	−.035±.039
Abdominal index	−.019±.039
Rear-chest index	+.004±.039

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[1] For explanation of item, see p. 209.

A comparison of this list with the two former lists shows that the correlation coefficients for length of intestines are definitely lower than those for either heart weight or lung weight; in fact, only 6 of 43 items have coefficients above 0.300 and can be classed as being even moderately significant. Width of thoracic cavity is near the top of the list, which is surprising since body widths were not significant in the other studies. The fact that five of the six items having coefficients above 0.300 are weights of thoracic organs or measurements of the thoracic cavity, is not easily explained.

Length of intestines does not appear to be significantly correlated with body weight or with the measurements of body bulk—not even volume of barrel. Moreover, it does not seem to be correlated with the external measurements of the paunch to any greater extent than with those of the chest. In fact, there is some indication that the reverse is true, though the differences are too slight and the coefficients too low to have much significance. Length of intestines is in no sense correlated with body scale, as body height and body lengths all have coefficients falling between 0.200 and 0.192. In this list, as in that for lung weight, the seven ratios and indexes representing body proportions are all located in the eight lowest places in the list. Attention is called to the fact that the ratios showing degree of wedge shape, which is commonly thought of as being associated with feeding capacity, all have coefficients ranging from 0.068 to 0.053.

These results cast considerable doubt on the possibility of efficiently judging the feeding capacity of cows on the basis of their external body measurements, for two reasons. One is the low magnitude of all the coefficients obtained in relation to length of intestines, and the other is the fact that the measurements of body mass, surface area, barrel capacity, body scale, and wedge shape nearly all have coefficients lower than those for measurements of thoracic cavity and weight of thoracic organs.

The correlation coefficients obtained for live weight in relation to other items are as follows:

	Live-weight coefficient
Range, 0.900 and over:	
Body-surface area	+0.939±0.004
Range, 0.800–0.899:	
Volume of barrel	+.838± .011
Circumference of fore chest	+.821± .012
Range, 0.700–0.799:	
Circumference of rear chest	+.788± .014
Weight of carcass	+.771± .015
Cross-section area of paunch	+.762± .015
Circumference of paunch	+.750± .016
Cross-section area of rear chest	+.745± .016
Depth of fore chest	+.721± .017
Cross-section area of fore chest	+.719± .017
Depth of rear chest	+.711± .018
Range, 0.600–0.699:	
Depth of paunch	+.680± .019
Width of paunch	+.676± .019
Weight of hide	+.664± .020
Width of hips	+.651± .020
Width of rear chest	+.650± .021
Volume of thoracic cavity	+.601± .026

	Live-weight coefficient
Range, 0.500–0.599:	
Length, withers to pinbones	+0.586±0.023
Width of fore chest	+.586± .023
Height at withers	+.572± .024
Weight of heart	+.509± .031
Length, withers to hips	+.508± .026
Weight of liver	+.504± .032
Range, 0.400–0.499:	
Width of loin	+.491± .027
Length (anterior-posterior) of thoracic cavity	+.486± .031
Weight of blood	+.484± .027
Weight of empty stomach	+.412± .032
Range, 0.300–0.399:	
Weight of empty intestines	+.376± .035
Weight of lungs	+.343± .039
Depth (dorsoventral) of thoracic cavity	+.325± .036
Width (lateral) of thoracic cavity	+.310± .037
Width of head	+.301± .032
Range, under 0.299:	
Abdominal index	−.275± .033
Length of intestines	+.268± .036
Length of loin	+.262± .034
Legginess	−.257± .033
Weight of spleen	+.247± .041
Rear-chest index	−.236± .034
Wedge shape, depth ratio [1]	+.236± .034
Thoracic index	.232± .034
Wedge shape, circumference ratio [1]	+.191± .034
Weight of pancreas	+.172± .040
Wedge shape, width ratio [1]	+.117± .035
Weight of brain	+.066± .038

[1] For explanation of item, see p. 209.

The coefficients obtained for many items when correlated with live weight are higher than anticipated. Among the 125 given in the three previous lists, for heart weight, lung weight, and length of intestines, only 3 were above 0.500 and could be considered as high. These three all pertain to heart weight. The maximum coefficient in the lung-weight list was 0.444 and that in the intestine-length list was only 0.387. Live weight was found to have correlation coefficients above 0.500 for 23 of the 44 items studied. Practically all of the 23 items, however, were items indicating body dimensions or body bulk. Only two were items showing size of internal organs—namely, the heart and the liver.

A large cow, particularly a bulky cow, is almost inevitably a heavy one. As a consequence, the measurements of mass or bulk would almost certainly have high coefficients of correlation with live weight, and it is not surprising to find body-surface area, volume of barrel, weight of carcass, and all the circumferences and cross-section areas of the fore chest, rear chest, and paunch in the highest sections of the list (all above 0.700). The location of body-surface area (0.939) at the top of the list is to be expected, since live weight is used as a factor in calculating body-surface area. The body depths all have coefficients of very high magnitude, ranging from 0.680 upwards, and the coefficients for body widths are much more significant in the live-weight correlations than in those for heart weight, lung weight, and intestine length.

The measurements of length and height, previously discussed as representing body scale, have rather highly significant coefficients (all above 0.500), whereas in the previous considerations, they were definitely less significant. The coefficients for measurements of thoracic cavity, although moderately or definitely significant, are more remote from the top in the live-weight list than in the other lists. Coefficients for these measurements are even lower than those for volume of thoracic cavity, which has the high coefficient of 0.601. This may appear to be contradictory, but it probably means that the total volume of the thoracic cavity increases with greater body size without regard to any given dimension of the thoracic cavity, that is, of length, width, or depth. The increased volume may come from an increase in one of these dimensions in some cases, from an increase in another dimension in other cases, or from an increase in all these dimensions in still other cases. None of the ratios or indexes is significantly correlated with live weight. Width of head and length of loin are not very significantly correlated with increased live weight, and legginess has a very moderately significant negative correlation.

As stated, the only internal organs having coefficients above 0.500 are weight of heart and weight of liver. Weights of stomach, intestines, and lungs have moderately significant coefficients, but length of intestines and weights of spleen, pancreas, and brain appear to have little relation to weight of body. In fact the brain has the lowest coefficient (0.066) of the 44 items.

One particularly important point is the fact that the single body measurement having the highest correlation coefficient (0.821) is circumference of fore chest, a measurement employed in Denmark and used for generations in some sections of the United States as a basis for estimating live weight in cattle. A compilation of data by the Bureau of Dairy Industry has provided a table [9] from which the live weight of dairy cattle can be estimated from the heart girth with a very high degree of accuracy. From this table a tapeline has been prepared which permits a direct reading of the live weight when the tapeline is passed around the body in the region of the fore chest. Similar tables [10] have since been prepared by the Bureau of Animal Industry for estimating the live weight of beef and dual-purpose cattle on the same basis. Attention is also called to a table for estimating live weight from heart girth, prepared by Ragsdale and Brody (*13*) and to one prepared by Brody, Davis, and Ragsdale (*2*), which includes the data obtained at the Missouri and Nebraska agricultural experiment stations.

In general, the body measurements—particularly those that represent bulk, magnitude, and scale—are highly correlated with live weight. The only internal organs whose weights are highly correlated with live weight are liver and heart. There is no relation between weight of brain and live weight. The coefficients for legginess and for relation of depth to width of body are all negative but of low magnitude. They do, however, suggest a slight tendency for the short-legged broad animal to have greater body weight than one with long legs and a deep narrow body.

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[9] KENDRICK, J. F., and PARKER, J B. ESTIMATING THE WEIGHTS OF COWS FROM HEART-GIRTH MEASUREMENTS. U. S. Bur. Dairy Indus. BDIM-695, 2 pp 1936 [Mimeographed]

[10] KNAPP, BRADFORD, JR A METHOD OF ESTIMATING THE WEIGHTS OF BEEF AND DUAL-PURPOSE CATTLE FROM HEART-GIRTH MEASUREMENTS. U. S. Bur. Anim. Indus. A. H. D. 24, 4 pp. 1937. [Mimeographed.]

COMPARISON OF CORRELATION COEFFICIENTS FOR HEART WEIGHT, LUNG WEIGHT, INTESTINE LENGTH, AND LIVE WEIGHT

In the discussions of the individual correlation coefficients presented in the foregoing lists, frequent reference has been made to the comparative magnitude and range of coefficients for different combinations of items. These combinations or groups differ according to the arrangement of the individual items in the list under consideration. A uniform method of grouping the different ante-mortem and post-mortem items was needed to permit a comparison of the extent to which the heart weight, lung weight, intestine length, and live weight of the cows studied was correlated with certain body dimensions and proportions (depths, widths, heights, etc.), and with the size of the organs and body parts that are associated with the various body functions. Accordingly, the ante-mortem items were divided into eight groups: (1) live weight, (2) body height, (3) body lengths, (4) body depths, (5) body widths, (6) body circumferences, (7) the calculated body-bulk values (body-surface area, volume of barrel, and the cross-section areas of fore chest, rear chest, and paunch), and (8) the calculated body-proportion values. The latter include legginess, thoracic, rear-chest, and abdominal indexes, and the ratios representing wedge shape as determined on the basis of depth, width, and circumference of body. The post-mortem items were classified in five groups: (1) body size, (2) nervous system, (3) circulation and respiration, (4) digestion, and (5) endocrine glands, as described in connection with the discussion of variation.

The mean or composite correlation coefficient for each group was determined as follows: The sum of the products obtained by multiplying each coefficient by the square root of its population was divided by the sum of the square roots of the populations, for all of the items included in the group. This is an empirical method of arriving at composite correlation coefficients for the respective groups, on a basis whereby each of the individual coefficients is weighted as to population. Although each of two of the ante-mortem classifications (live weight and body height) and one of the post-mortem groups (nervous system) contain only a single item, they are referred to as groups and the individual coefficient is referred to as a composite. A summary of the composite correlation coefficients for each ante-mortem and post-mortem group is given in table 3.

As an illustration of the data presented in table 3, it will be noted in the column under heart weight that live weight, which represents only one item, has a coefficient of correlation of 0.509. Since this is the highest coefficient under heart weight it is given a rank of 1. The second highest coefficient, 0.472, is that of body circumferences. This coefficient represents a composite of those for three items in the list of heart-weight correlations on page 215, as follows: Circumference of fore chest, coefficient 0.494; circumference of rear chest, coefficient 0.484; and circumference of paunch, coefficient 0.437. The third highest in rank of the composite coefficients for heart weight is that for the calculated body-bulk values, and the fourth highest is that for body depths. At the other extreme, the thirteenth or lowest composite coefficient of correlation with heart weight is that for the calculated body-proportion values; the twelfth is that for the nervous-system group, represented by the brain; the eleventh is that for the digestion group; and the tenth is that for body lengths. It appears, therefore, that the items most likely to be associated with hearts of greater weight in cows are those which indicate body magnitude such as live weight, calculated body-bulk values, body circumferences, and body depths.

TABLE 3.—*Summary of the composite correlation coefficients for each functional group in each list and for all lists combined*

Group	Items in group	Heart weight		Lung weight		Intestine length		Live weight	
		Rank	Coefficient	Rank	Coefficient	Rank	Coefficient	Rank	Coefficient
Ante-mortem:	*Number*								
Live weight	1	1	0.509	5	0.343	2	0 268	------	--------
Body height	1	8	.364	7	.303	9	.200	4	0.572
Body lengths	[1] 2	10	.302	1	.430	11	.186	7	.454
Body depths	3	4	.429	2	.410	3	.258	3	.704
Body widths	[2] 3	9	.329	11	.208	10	.188	5	.559
Body circumferences	3	2	.472	6	.338	7	.244	2	.786
Calculated body-bulk values	5	3	.450	3	.366	8	.230	1	.801
Calculated body-proportion values	7	13	.079	13	.048	13	.044	11	.220
Post-mortem:									
Body-size group	6	7	.412	4	.353	1	.287	6	.535
Nervous-system group	1	12	.124	12	.160	12	.061	12	.066
Circulation-and-respiration group	[3] 4	5	.423	9	259	5	.251	8	.449
Digestion group	[4] 4	11	.272	10	.238	6	.250	9	.345
Endocrine-gland group	1	6	.415	8	.295	4	.255	10	.247
Composite for all items represented [5]	------	------	.335	------	.270	-----	.202	------	.502

[1] Length of loin added under intestine length and live weight.

[2] Widths of loin and hips added under intestine length, widths of loin, hips, and head added under live weight.

[3] Heart weight omitted under heart weight, lung weight omitted under lung weight; heart circumferences omitted under intestine length and live weight

[4] Intestine length omitted and intestine weight added under intestine length, intestine weight added under live weight.

[5] Total number of items represented under heart weight, 41, under lung weight, 41; under intestine length, 43; and under live weight, 44.

Since heart weight has a high correlation with live weight, it is to be expected that the items which are indicative of heart weight would also be indicative of live weight, and for the most part this is true. There is considerable similarity in the ranking of the coefficients under the two headings, although a number of exceptions exist. The endocrine-gland group, which is represented by only one organ, the spleen, is more closely associated with heart weight than with live weight. The circulation-and-respiration group also has a higher rank in the heart-weight than in the live-weight column, probably because the heart circumferences are included in the former but not in the latter. On the other hand, the measurements of body height, body widths, and body lengths have higher ranks and are more indicative of live weight than they are of heart weight. The items in the digestion, nervous-system, and body-proportion groups seem to be a little more closely related to live weight than to heart weight. This is also true of their relationship to lung weight and to intestine length, except that the rank of the digestion group is highest in the intestine-length column. This is no doubt due in part to the fact that the intestine-length and intestine-weight coefficients are included in the group.

Many of the composite coefficients in the lung-weight column and most of the coefficients in the intestine-length column are too small to be significant. The great difference in relative ranking of some of the coefficients in these two columns from those in the heart-weight and live-weight columns, is interesting and perhaps significant. For instance, the composite coefficients for body lengths rank first under lung weight but have a low or medium rank under the other three heads. The composite coefficients for the body-depth measurements rank second for lung weight and third for intestine length. These ranks are equal to or higher than those for heart weight and live weight. Body circumferences, however, which ranked second with heart weight and with live weight, dropped to sixth and seventh with lung weight and intestine length. Body widths appear to be of comparatively little importance in relation to heart weight, lung weight, or intestine length but have a definitely higher correlation for live weight. Live weight and the post-mortem body-size group rank high among the coefficients for intestine length. Too much significance should not be placed on the relative ranking of these composite coefficients under intestine length, however, because of their low values. For instance, the highest ranking coefficient under intestine length is that for the body-size group, with a value of 0.287. The coefficient for this same group ranks seventh for heart weight and sixth for live weight, but has values of 0.412 and 0.535, respectively. Therefore, these sixth or seventh place coefficients of this group must be more indicative of heart weight and live weight than is the coefficient having first rank for this same group under intestine length.

It is difficult to explain why the live-weight group has a rank of 5 for lung weight, whereas for heart weight and intestine length its rank is 1 and 2, respectively. Lung weight appears to be more closely correlated with several of the skeletal measurements than with body weight.

The calculated body-bulk values (ante-mortem) are, as might be expected, closely correlated with live weight (rank 1). Both heart weight and lung weight also rank high for this group but length of intestines has a rank of 8, with the very low composite of 0.230.

The comparatively high ranks (2 to 4) for body depths in all cases may point to a comparatively high significance of body-depth measurements and their importance as a measure of body size and as an indicator of size of internal organs.

Body height—one of the measurements of scale—is fairly closely correlated with live weight, but has not only low coefficients but lower ranks (8, 7, and 9, respectively) for heart weight, lung weight, and intestine length.

The body widths are definitely less closely correlated with live weight than are body depths. The difference between the ranks for depth and for width is less for live weight than for heart weight, lung weight, or intestine length. Probably this is because an increase in width of body has a tendency to contribute directly to live weight through fat deposition, whereas depth of body is more nearly a measurement of skeletal size.

Some of the facts concerning the post-mortem, body-size group are not readily explainable. This group includes the weight of hide, weight of carcass, and the three dimensions and the calculated volume of the thoracic cavity. Four of the six items are measurements of the thoracic cavity. Although the composite coefficient for this group listed under live weight, table 3, is of fairly high magnitude, it ranks sixth in the live-weight list despite the tendency for weight of carcass to raise the composite value. Apparently the size of chest cavity is not so closely correlated with live weight as are a number of the external body measurements that are more representative of body mass than of skeletal size. This is more specifically shown by the fact that the composite coefficient for the four thoracic measurement items alone in relation to live weight is 0.430. This value is lower than any of those listed in table 3 for the ante-mortem groups in the live-weight list, except the one for calculated body-proportion values. Moreover, composite coefficients based on the four thoracic measurement items considered as a group in relation to heart weight, lung weight, and length of intestines, are 0.390, 0.372, and 0.310, respectively. This fact indicates that the size of the heart is not as closely correlated with the size of the cavity in which it is located as with the ante-mortem groups for live weight, body depths, body circumferences, and calculated body-bulk values, or with three of the post-mortem groups, whereas the weight of the lungs, which also are housed in the thoracic cavity, is more highly correlated with the size of that cavity than with any other groups of external body or internal anatomical measurements except lengths and depths of body. Strangely, the composite coefficient of 0.310 for the four thoracic measurements in relation to length of intestines, which are located in the abdominal cavity, is higher than the composite for any of the ante-mortem or post-mortem groups listed in table 3 under intestine length.

Composite coefficients listed under live weight in table 3 are much higher for the ante-mortem than for the post-mortem groups, indicating that the external body measurements are more closely correlated with live weight than are the size of the internal organs or the measurements of the thoracic cavity. There is a tendency also for the composites listed under heart weight and lung weight to be of comparatively higher magnitude in the case of the ante-mortem groups, but the difference is negligible for length of intestines.

Composite coefficients shown in table 3 for the 41 heart-weight items, the 41 lung-weight items, the 43 intestine-length items, and the 44 live-weight items, indicate that on an average live weight is rather highly correlated, heart weight is moderately correlated, and lung weight and intestine length have little relation to body dimensions and anatomy in cows.

COMPARISON OF CORRELATION COEFFICIENTS FOR DEPTHS AND FOR WIDTHS OF FORE CHEST, REAR CHEST, PAUNCH, AND THORACIC CAVITY

Although by no means the only result brought out or necessarily the one having the greatest importance from a practical viewpoint, one of the most consistent results shown is the comparatively high magnitude of correlation coefficients obtained for depths as compared with widths of body in relation to heart weight, lung weight, intestine length, and live weight. Because of the consistency of this result and the fact that similar results were reported by Becker (*1*), Seeberger (*15*), and Mierswa (*8*), it seemed desirable to determine additional correlations and to test the consistency of the data when different combinations and greater numbers of items of external body dimensions and internal anatomy were included.

In the case of the fore-chest measurements, comparable coefficients have been determined for 11 items, and coefficients not strictly comparable are given for 2 other items. Composite coefficients also have been calculated for all groups which contain more than one item having comparable coefficients for depth and width. Since two comparable coefficients of correlation are given for each of the selected items, it was not possible in both cases to arrange the items in the order of descending magnitude of the coefficients. The items were grouped for convenience of comparison according to the previously used classification (pp. 222–223) based on the dimensions of the body and the functions of its parts. The results are shown in table 4.

TABLE 4.—*Comparison of the individual and group composite correlation coefficients for depth and for width of fore chest*

Item	Coefficient of correlation and probable error, for—		Extent to which coefficient for depth exceeds that for width
	Depth of fore chest	Width of fore chest	
Ante-mortem:			*Percent*
Live weight	0.721±0.017	0.586±0 023	23
Body height (at withers only)	.658± .020	.251± .033	162
Body-length group:			
Withers to hips	722± .017	.106± .035	581
Withers to pinbones	.645± .021	.201± .034	221
Composite for group	684	153	347
Body circumference (at fore chest only)	796± .013	659± .020	21
Post-mortem.			
Body-size group:			
Weight of hide	.627± .022	463± .028	35
Depth (dorsoventral) of thoracic cavity	[1] .611± 026		
Width (lateral) of thoracic cavity		[1] 423± 034	
Volume of thoracic cavity	.644± .024	295± .038	118
Composite for group	.635	385	65
Circulation and respiration group:			
Weight of blood	.446± .028	.296± 032	51
Weight of lungs	432± 036	200± .042	116
Weight of heart	.436± 034	315± .038	38
Composite for group	.439	.273	61
Digestion group:			
Length of intestines	.252± 037	.122± 039	107
Composite for all comparable items	.587	.322	82

[1] Not comparable.

The coefficient for depth of fore chest exceeds the corresponding one for width of fore chest in the case of every one of the 11 individual items and of each group compared, confirming observations previously made. This excess is expressed as a percentage of the value of the width coefficient, in the last column of table 4. The relative degree of excess varies from 581 percent for length from withers to hips, to 21 percent for circumference of fore chest. It is noteworthy that the three highest individual differences all appear in connection with the items representing body height and length. Since these differences are the result of high coefficients for depth and low coefficients for width, they indicate that depth of body (fore chest) increases along with increases in height and length of body, whereas width of body does not follow so closely. The two lowest differences are found in connection with live weight and circumference of fore chest—both measurements of body mass. All of the coefficients involved in this case are of high magnitude. Width of chest follows increases in live weight and chest circumference more closely than it follows increases in skeletal measurements. It appears that fleshing affects width of chest to a greater extent than it affects depth of chest. Length of intestines is not significantly correlated with either depth or width of fore chest. It appears that the measurements of body scale are highly correlated with those of depth but not with those of width of fore chest, that the size of the internal organs is not highly correlated with either depth or width of fore chest, and that the measurements of body mass (circumference of fore chest and live weight) are highly associated with both depth and width of fore chest.

In view of the high correlation between body (fore chest) width and circumference, the much lower coefficient for body width with weight of hide, which probably follows body surface quite closely, is rather surprising. This might be explained by the low correlation of body width with body length, which would be the other dimension in determining body surface, or by variations in the thickness of hide. The correlation between the exterior depth of chest and depth of thoracic cavity is high. The correlation between the exterior width of chest and width of thoracic cavity is significant, but is much lower than that for the corresponding depths. The correlation between exterior depth of chest and volume of thoracic cavity is even higher than that between the exterior depth and the depth of the cavity, but the correlation between exterior width and volume of the thoracic cavity is much lower than that between exterior width and interior width. This probably indicates that for thoracic cavity, length is a more important factor than width in determining its volume. It may also explain the relatively high correlation shown between body lengths and lung weight (table 3) and between length of thoracic cavity and lung weight (p. 216).

In order to determine whether the same ratio or percentage of difference between coefficients for depth and width that was shown for measurements of the fore chest would prevail in connection with measurements of the rear chest, paunch, and thoracic cavity, coefficients of correlation for these additional measurements with heart weight, lung weight, intestine length, and live weight were calculated. The results are given in table 5.

The depth (dorsoventral) and width (lateral) measurements of the thoracic cavity were taken at a plane intermediate between the planes used in obtaining external measurements of depth and width of fore chest and rear chest. Depth and width of thoracic cavity are particularly suitable for study because their accuracy was not, like some ante-mortem measurements, dependent on the standing position of the animal. The carcasses were always in the same position (suspended) when the measurements were obtained. Moreover, the thoracic measurements are virtually skeletal dimensions of the chest cavity and are not greatly affected by fleshing or fat deposition.

TABLE 5.—*Comparison of the correlation coefficients for depth and for width of fore chest, rear chest, paunch, and thoracic cavity*

Item	Fore chest			Rear chest		
	Depth coefficient	Width coefficient	Relation of width to depth coefficient	Depth coefficient	Width coefficient	Relation of width to depth coefficient
			Percent			*Percent*
Live weight	0.721	0.586	81.3	0.711	0.650	91.4
Heart weight	.436	.315	72.2	.457	.348	76.1
Lung weight	.432	.200	46.3	.418	.211	50.5
Intestine length	.252	.122	48.4	.287	.176	61.3
Composite coefficient	.469	.316	67.4	.476	.358	75.2

Item	Paunch			Thoracic cavity		
	Depth coefficient	Width coefficient	Relation of width to depth coefficient	Depth coefficient	Width coefficient	Relation of width to depth coefficient
			Percent			*Percent*
Live weight	0.680	0.676	99.4	0.325	0.310	95.4
Heart weight	.392	.324	82.7	.427	.321	75.2
Lung weight	.379	.214	56.5	.383	.302	78.9
Intestine length	.236	.173	73.3	.250	.353	141.2
Composite coefficient	.431	.360	83.5	.344	.322	93.6

All four individual items have coefficients which are greater for depth than for width of fore chest, of rear chest, and of paunch, although the difference for live weight in the latter comparison is too small to be of any significance. Correlation coefficients are greater for depth than for width of thoracic cavity for three of the four items compared, the only exception occurring in connection with length of intestines. A study of the weight of heart and weight of lungs (both thoracic organs) in relation to the depth and width of fore chest, rear chest, and thoracic cavity (all thoracic measurements) shows composite coefficients of 0.434 and 0.259, respectively, for depth and width of fore chest; 0.438 and 0.281 for depth and width of rear chest; and 0.405 and 0.312 for depth and width of thoracic cavity. Although the difference is not as great for the thoracic cavity as for the external chest measurements, it is sufficiently great to indicate a definite tendency for the thoracic organs to be more highly correlated with depth than with width of chest.

There is a definite tendency for the difference in the magnitude between the correlation coefficients for depth and for width to be less for measurements at the rear chest than at the fore chest and still less for those at the paunch, when these measurements are studied in relation to live weight, heart weight, lung weight, and intestine length.

With respect to body measurements, in the case of live weight the correlation coefficient for width of body is 81.3 percent as great as that for depth measured at the fore chest, 91.4 percent at the rear chest, and 99.4 percent at the paunch. In the case of heart weight, the coefficient for width is 72.2 percent as great as that for depth when measured at the fore chest, 76.1 percent as great when measured at the rear chest, and 82.7 percent as great at the paunch. The corresponding percentages are 46.3, 50.5, and 56.5, respectively, for lung weight, and 48.4, 61.3, and 73.3, respectively, for intestine length. These differences appear to be too great to be accounted for by changes that might be brought about by the position in which the animal was standing when measured, or by differences in deposition of fat.

The coefficients of correlation for live weight with thoracic-cavity depth and width in table 5 are less than half as great as those for the exterior body measurements, and the coefficient for width is almost as great as that for depth. In respect to heart weight, the coefficients of correlation with measurements of the thoracic cavity do not differ greatly in magnitude from those obtained for the exterior measurements, and the relationship of the coefficient for width of thoracic cavity to that for depth is about the same as those for the fore-chest and rear-chest measurements. In the correlation of lung weight with thoracic cavity, the coefficient for width is greater than the width coefficient for any of the three exterior measurements, and the percentage relationship of the coefficient for width of thoracic cavity to that for depth of the cavity is much greater than that for any of the exterior depths and widths. In the correlations with intestine length, the coefficient for depth of thoracic cavity is not greatly different from those for depth of fore chest, rear chest, and paunch, but the coefficient for width of thoracic cavity is much greater than that for any other depth or width in the series for intestine length, and is the only one that is large enough to be significant. The coefficient for width of thoracic cavity is 141.2 percent as great as that for depth of thoracic cavity.

RESULTS FOR THE DIFFERENT BREEDS

Up to this point the study has dealt with the combined data for all breeds. Analysis of the data in this way, although not ideal, seemed warranted, inasmuch as many of the cows could not be definitely identified with any specific breed and to divide those remaining into groups would too greatly limit the number of data for some of the breeds. However, to determine whether the results obtained by studying the combined data were representative for the different breeds, the data were divided into breed groups. Only those cows that showed definite characteristics of some breed were used in this phase of the study. On account of the small number of animals considered to be Jerseys, Guernseys, and Ayrshires, and their similarity in size, the cows of these three breeds were combined in one breed group to be compared with the Holsteins and the Shorthorns, which appeared in fairly large numbers. Correlation coefficients for depth of fore chest and for width of fore chest with heart weight and lung weight are presented in table 6.

There appear to be some significant differences in the breeds as grouped in table 6. In the coefficients of correlation for heart weight with depth and width of fore chest, the coefficient for width of fore chest is 92.7 percent as great as that for depth of fore chest in the Holsteins, but it is only 55.2 percent as great in the Shorthorns, and 64.5 percent as great in the Ayrshire-Guernsey-Jersey group. The correlation between heart weight and depth of fore chest is highest for the

Ayrshire-Guernsey-Jersey group and lowest for the Holsteins. The correlation between heart weight and width of fore chest is greatest for the Holsteins and least for the Shorthorns.

Table 6.—*Correlation coefficients for depth and width of fore chest, studied in relation to heart and lung weight, and divided by breeds*

Item	Holstein				Shorthorn				Ayrshire-Guernsey-Jersey			
	Animals compared	Coefficient for depth of fore chest	Coefficient for width of fore chest	Relation of width to depth coefficient	Animals compared	Coefficient for depth of fore chest	Coefficient for width of fore chest	Relation of width to depth coefficient	Animals compared	Coefficient for depth of fore chest	Coefficient for width of fore chest	Relation of width to depth coefficient
	Number			*Percent*	*Number*			*Percent*	*Number*			*Percent*
Weight of heart	114	0.450	0.417	92.7	54	0.460	0.254	55.2	45	0.519	0.335	64.5
Weight of lungs	97	.362	.217	59.9	47	.364	.324	89.0	44	.489	.229	46.8

In the correlations between lung weight and depth and width of fore chest, the coefficient for width is 59.9 percent as great as that for depth in the Holsteins, 89.0 percent as great in the Shorthorns, and only 46.8 percent as great in the Ayrshire-Guernsey-Jersey group. The correlation between lung weight and depth of fore chest is greatest for the Ayrshire-Guernsey-Jersey group; coefficients for the Shorthorns and the Holsteins are much lower and on very nearly the same level. The correlation with width of fore chest is greatest for the Shorthorns; coefficients for the Ayrshire-Guernsey-Jersey and Holstein groups are considerably lower and on approximately the same level. No explanation is offered for these apparent differences in the breed groups. It seems possible that they would be modified to some extent if larger numbers were available for the correlations.

Since the breed study was undertaken primarily to determine whether or not the breed data followed the pattern of the combined data, reference is made to the results of that comparison. Although a number of breed differences are apparent, the coefficient for depth is greater than the corresponding one for width of fore chest in each of the six comparisons shown in table 6. Moreover, the heart-weight coefficient exceeds the corresponding lung-weight coefficient in five of the six comparisons. Composite coefficients for heart and for lungs are 0.409 and 0.325 respectively. Comparative composites for heart weight and for lung weight based on the combined data were 0.376 and 0.316 when only the measurements of fore chest were included, and 0.335 and 0.270, respectively, when the comparison was based on all of the 40 items studied in relation to weight of heart and lungs. Despite the breed differences noted, therefore, the results obtained were generally similar whether the study was based on data for separate breed groups or on the combined data from all of the animals.

SUMMARY

The results here reported are confined to studies of the relationship between external form and internal anatomy since information regarding the producing capacity of the cows included in this study was not available.

In addition to live weight and thickness of hide, the ante-mortem items studied include 17 measurements of skeletal size, 5 calculated values representing body bulk, and 10 calculated values showing body proportions. The post-mortem items include 19 weights or measurements showing size of organ or body part, 2 ratios showing the relation of heart and lung weight to live weight, and 1 value calculated to give an approximate measure of the volume of the thoracic cavity.

In both the ante-mortem and post-mortem data the arithmetic average and the median are almost equal for each individual item, indicating a normal distribution of data centering close to the average. The cattle selected for study apparently constitute a representative sampling.

The coefficients of variation for the calculated values representing body bulk are on an average greater than for any group of actual measurements of skeletal dimensions. The calculated body-proportion values show an even greater average variation; of these, the coefficients for wedge shape based on differences in measurement of fore chest and paunch have the highest average. The greatest variation in the group representing skeletal size is for length of loin, followed by the five measurements for width of body. On an average, variations for the ante-mortem measurements of skeletal size are much greater for width than for circumferences, depths, lengths, or height of body. Aside from the great variation in thickness of hide and in length of loin, the greatest variations are associated with measurements most likely to be affected by condition of fleshiness and fill, and the least with measurements representing skeletal size or body scale. The highest average variations for the post-mortem items occur in the group of visceral fats, the second highest in the group of digestive organs, and the lowest in the nervous-system group, represented by the brain, and the body-size group, which includes weight of hide, weight of dressed carcass, and carcass measurements. The pancreas has the highest individual variation and the three measurements of the thoracic cavity the three lowest. The coefficients for the heart and lungs are much larger than those for the individual dimensions of the thoracic cavity. Variations both for the individual items and for functional groups are much greater for the post-mortem than for the ante-mortem items. It appears that even with animals of the same body size and shape there may be marked differences in internal anatomy.

A total of 169 individual correlation coefficients have been determined to show the relation of heart weight, lung weight, intestine length, and live weight to other ante-mortem and post-mortem items and to each other. Additional determinations show the relation between depth and width of fore chest, rear chest, paunch, and thoracic cavity, and certain individual ante-mortem body dimensions and post-mortem weights and measurements. These studies are based on the combined data from all the animals included in the study. A separate study, in which the data are separated into breed groups, has been made to determine to what extent some of the results obtained from analyzing the combined data are supported by the data for the different breeds.

Heart weight is rather highly correlated with live weight and with the measurements associated with body bulk, such as body-surface area, volume of barrel, body circumferences, weight of carcass, and weight of hide. Heart weight is moderately correlated with depth of chest, and with depth, length, and volume of the thoracic cavity in which it is located, but it appears to be only slightly correlated with width of body, width of thoracic cavity, or with height and length of body, which represent body scale. Only moderate correlations exist between heart weight and the weight or measurement of other internal organs except weight of liver, and for several of them (weights of pancreas, stomach, and brain) the correlations are of no significance. Heart weight is not significantly correlated with any of the ratios or indexes calculated to represent body proportions.

In general the individual correlation coefficients for lung weight are definitely lower than those for heart weight. The lung-weight correlations, like the heart-weight correlations, are comparatively high for volume of barrel and body-surface area, but correlations for the other body-bulk items are at best only moderate. A fairly significant correlation appears to exist between weight of lungs and the depth, length, and volume of the thoracic cavity in which they are located. Body lengths not only appear near the top of the list of lung-weight correlations, but their coefficients are actually greater in magnitude than those for heart weight. Despite this fact, body height -- another measurement of body scale--has an even lower coefficient for lung weight than for heart weight. The lung weight has a much lower coefficient than the heart weight (0.343 and 0.509 respectively) when correlated with live weight. Lung weight is definitely more highly correlated with depth than with width of body or of the thoracic cavity. There is no significant relation between lung weight and the ratios and indexes that indicate body proportions. Aside from moderate coefficients for heart and liver weights, there is no significant correlation between weight of lungs and the size of other internal organs. Lung weight cannot be considered as highly correlated with either the ante-mortem or the post-mortem items studied, though some moderately significant coefficients are shown.

The correlation coefficients are definitely lower for length of intestines than for either heart weight or lung weight, only 6 of the 43 items studied being above 0.300. Length of intestines does not appear to be significantly correlated with body weight or the measurements of body bulk—not even volume of barrel. There is no significant correlation for the items representing body scale or body proportions, although the latter include wedge shape which is commonly thought of as being associated with capacity for feed consumption. Length of intestines appears to be more highly correlated with external measurements of the chest than with those of the paunch, although the differences are too small to have much significance. Most surprising of all findings is the fact that intestine length is more closely correlated with the weights of the thoracic organs and the width, volume, and length of the thoracic cavity, than with live weight, body bulk, body scale, or with abdominal or any other body measurements. This fact, together with the low magnitude of all of the intestine-length coefficients, casts considerable doubt on the possibility of efficiently judging the feeding capacity of cows on the basis of their external conformation.

Comparatively high correlations were anticipated for live weight, but the magnitude of the coefficients exceeded expectations. Among the 125 coefficients for heart weight, lung weight, and intestine length only 3 were above 0.500, yet 23 of the 44 live-weight coefficients were above that level. Practically all of these, however, were for items indicating body dimensions or body bulk, only 2 of the 23 items being for internal organs. The highest coefficients are found in connection with body-surface area and volume of barrel, and nearly all the measurements of body bulk have coefficients above 0.700. The single body measurement having the highest coefficient (0.821) is for circumference of fore chest or "heart girth," a measurement employed for generations in Denmark and in some sections of the United States as a basis for estimating live weight in cattle. Body widths have lower coefficients than body depths though both are high. Measurements of body scale also are rather highly significant. Volume of thoracic cavity is highly correlated and length of thoracic cavity is more definitely correlated with live weight than either depth or width, though all the dimensions of the cavity are less significantly correlated than is volume. The only internal organs whose weights are highly correlated with live weight are heart and liver. There is no relation between weight of brain and live weight. Degree of wedge shape does not appear to be associated with live weight. The live-weight coefficients indicate that a heavy animal is almost certain to have, as is to be expected, a large surface area, a large volume of barrel, and large circumferences. Such an animal may be expected to have a correspondingly large heart and liver, and will also probably have a large quantity of blood, large stomachs, large lungs, and heavy intestines. There is little likelihood that the intestines or the loin will increase in length proportionately, and very little probability that any marked relationship will exist between live weight and weights of spleen, pancreas, or brain.

When the individual ante-mortem and post-mortem items were uniformly grouped on the basis of body dimensions or function of internal organs and body parts, the composite correlation coefficients for the various groups showed a wide range of variation in magnitude. The ranking of the groups on the basis of the magnitude of their composite coefficients is in some respects similar for heart weight, lung weight, and intestine length to that for live weight. One of the most noteworthy differences is the very high rank of body lengths for lung weight. In fact lung weight appears to be correlated more closely with a number of the skeletal measurements than with live weight. Other striking differences are the low rank of the body-bulk values, and the very high rank of the body-size group consisting largely of thoracic measurements, for intestine length. Body circumferences have high ranks for heart weight and live weight but lower values for lung weight and intestine length. The endocrine-gland group (spleen) has a very low rank for live weight though the coefficient is only slightly lower than for intestine length, where it ranks fourth. Ranks for body-proportion values and for brain weight are consistently low. Those for body widths are low except for live weight. Body depths have consistently high ranks—a fact which may point to the significance of depth of body as one of the most reliable indications of live weight and of size of internal organs.

Size of thoracic cavity does not appear to be as closely correlated with live weight as are a number of the external body measurements that are more representative of body mass than of skeletal size. The weight of heart is not as closely correlated with the size of the thoracic cavity as live weight and many of the external body measurements. The weight of lungs, however, is more highly correlated with size of thoracic cavity than with any other group except lengths and depths of body. Length of intestines is more highly correlated with measurements of the thoracic cavity than with any of the ante mortem or post mortem groups.

It appears that, in general, live weight is rather highly correlated, that heart weight is moderately correlated, and that lung weight and intestine length have comparatively little relation to body dimensions and anatomy in cows.

The measurements of body scale (height and length of body) appear to be highly correlated with those for depth, but not with those for width of fore chest; the size of the internal organs is not highly correlated with either depth or width of fore chest, and the measurements of body mass (circumference of fore chest and live weight) are highly correlated with both depth and width of fore chest.

The correlation for exterior depth of chest and volume of thoracic cavity is high, even higher than that between the exterior depth of chest and depth of the cavity, but the correlation between exterior width and volume of the thoracic cavity is much lower than that for the exterior width and the interior width, probably indicating that length of the thoracic cavity is a more important factor in the volume of the thoracic cavity than is width.

In correlations of depth and width of fore chest, rear chest, and paunch with live weight, heart weight, lung weight, and intestine length, the magnitude of the correlations for width, as compared with those for depth, increases as the measurements progress from fore chest to paunch.

The data were divided into three breed groups: (1) Holsteins, (2) Shorthorns, and (3) Jerseys, Guernseys, and Ayrshires to determine whether or not certain results obtained with the combined data would hold for breeds differing widely in size and form. Because of the consistently marked difference between correlations for depth and width of chest these correlations were used as a basis for testing the data. While the results of the breed study substantiate those obtained from analysis of the combined data in showing a greater magnitude of correlation coefficients for depth than for width of fore chest, and higher coefficients for heart than for lungs, the study appears to indicate that there may be some significant breed differences. These suggested differences will be further explored when the more numerous data for the different breeds now being obtained for the purpose of determining the relationship of external form and size of internal organs to producing ability, are analyzed.

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(4) *G. tomentosum* Nutt. (Hawaiian Islands) and (5) *G. taitense* Parl. (Fiji Islands, etc.).

EARLY HISTORY OF THE CYTOGENETICS OF GOSSYPIUM

Interspecific hybrids in *Gossypium* were made as early as 1790 by Rohr (Watt (*31*)), who recognized the possibility of improving the cotton crop by means of hybridization. In 1872 Clark (Watt (*31*)), reported that it was fairly easy to cross species of cotton within the Asiatic group or within the cultivated American group but that it was impossible to produce hybrids between the groups. Until 1923 Clark's report was generally accepted as correct, since all attempts to produce a hybrid between an Asiatic and an American species had resulted in failure. On the other hand, F_1 hybrids within the Asiatic and the cultivated American group, respectively, were found to be easily made and highly fertile and consequently capable of utilization in the improvement of cotton.

In 1923 Zaitzev (*39*) found the first recorded hybrid between a cultivated Asiatic and a cultivated American cotton and reported that it was completely sterile. In 1927 he (*40*) reported an artificially made Asiatic-American hybrid and described five natural hybrids between members of these groups. In the same year Desai (*11*) obtained one hybrid from numerous crosses between species of the two groups. Recently approximately 20 artificial and natural Asiatic-cultivated American hybrids have been reported by Zhurbin according to Emme (*12*), Nakatomi (*22*), Harland (*16*), Szymanek and Gavaudan (*30*), Baranov (*4*), Longley (*21*), Feng (*13*), and Skovsted (*25*).

Cytological investigations of *Gossypium* began in 1903 with Cannon's studies of the cultivated American hybrid *G. barbadense* × *G. hirsutum*. He (*6*) reported that the hybrid had 28 haploid chromosomes and that it exhibited complete chromosome conjugation and normal disjunction during the heterotypic division. Later Ball (*2*) reported *G. barbadense* to possess 20 haploid chromosomes. In 1923 and 1924 Nikolajeva (Zaitzev (*39*)) and Denham (*9*, *10*) established 13 as the haploid chromosome number for Asiatic species of *Gossypium* and 26 as the haploid chromosome number for the cultivated American species. At the same time Denham (*10*) reported complete chromosome conjugation in two Asiatic hybrids, *G. cernuum* × *G. rudicum*[3] and *G. arboreum* × *G. neglectum*. That all cultivated or wild Asiatic species have 13 pairs and all cultivated American species have 26 pairs of chromosomes has been confirmed by Youngman and Pande (*38*), Longley (*21*), Beal (*5*), Banerji (*3*), and Webber (*33*). Harland (*15*), Longley (*21*), and Webber (*33*) have found that *G. sturtii* and all wild American species of *Gossypium* have 13 haploid chromosomes.

In 1930 Baranov (*4*) reported that a cultivated American-Asiatic hybrid (*Gossypium hirsutum* × *G. herbaceum*) formed 13 bivalent and 13 univalent chromosomes during meiosis. He also observed that the univalents were distributed irregularly. Similar observations were reported in other cultivated American-Asiatic hybrids by Nakatomi (*22*) in 1931, and Zhurbin as reported by Emme (*12*) in 1930. Longley (*21*) reported variable chromosome conjugation in a natural hybrid of this type.

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[3] Name as given in Denham's paper, probably a misprint for *G. indicum* Lam. The latter is supposed to be the same as *G. nanking* Meyen.

SUMMARY OF RECENT CYTOGENETIC STUDIES OF GOSSYPIUM

Since 1934 there have been produced intergroup hybrids between species representing each of the four groups of *Gossypium* and between most of the distinctly different species within each of the groups. Most of these hybrids have been studied cytologically. The important interspecific hybrids and a few variant plants that have bearing on the phylogeny of the genus are listed in table 1, together with their reported meiotic chromosome pairing and fertility. The names of species as given in the table, are, for the most part, those under which the original seeds were received. It is very doubtful that many of the "species" that have been described in the cultivated Asiatic group and in the cultivated (and semiwild) American group are really entitled to this rank.

CHROMOSOME MORPHOLOGY

Skovsted (*25*) divided the species of *Gossypium* with 13 pairs of chromosomes into a group possessing large somatic chromosomes and a group having small somatic chromosomes. He placed *G. sturtii* in the group characterized by larger somatic chromosomes. Later he (*26*) depicted both the somatic and meiotic chromosomes of *G. sturtii* and reported that its meiotic chromosomes also were larger than those of species belonging to the group with smaller somatic chromosomes. According to the observations of the writer, the somatic chromosomes of *G. sturtii* are larger than those of the majority of species of *Gossypium* with 13 pairs of chromosomes, but the meiotic chromosomes are not correspondingly large.

According to Baranov (*4*), the somatic chromosomes of the cultivated Asiatic species exhibit such morphological distinctions as differences in length of arms, divided and undivided arms, arms with or without satellites, etc. Later Skovsted (*23*, *25*) reported that the Asiatic species are characterized by 13 pairs of relatively large chromosomes and that the 26 somatic chromosomes of *Gossypium arboreum* are all of equal size and 4 of them possess satellites. After a careful study of *G. herbaceum*, Arutjunova (*1*) divided the somatic complement into 13 types, noting that several of the chromosome types resembled one another and that 4 of them were much smaller than the others. Both Denham (*10*) and Davie (*8*) observed that one pair of chromosomes in the Asiatic cottons is longer than the others, and Davie found that the species of this group exhibited considerable secondary pairing. The writer agrees that the Asiatic species form a group characterized by 13 pairs of relatively large chromosomes but has found that the somatic chromosomes of Asiatic species are slightly smaller than those of *G. sturtii*. Neither Skovsted nor Arutjunova found the larger pair of chromosomes reported by Denham and Davie, and neither Webber (*33*) nor Skovsted has found secondary association in any Asiatic species.

Skovsted (*25*) classified the wild American species of *Gossypium* as a group having 13 pairs of smaller chromosomes. He (*26*) figured the somatic chromosomes of *G. davidsonii* as approximately one-half the length and one-half the diameter of those of *G. sturtii*, and later (*29*) found that secondary pairing in the wild American species is more noticeable than in other species with 13 pairs of chromosomes.

TABLE 1.—*Summary of the meiotic chromosome conjugation and fertility of interspecific hybrids and variants in Gossypium*

Category of hybrids, whether within a group or between groups, and haploid chromosome numbers of the parent species	Hybrid or variant	Meiotic chromosome behavior [1]	Fertility [2]	Investigator and reference
CATEGORY 1 Asiatic ($n=13$) × Asiatic ($n=13$). (Group 2 × group 2)	*G. arboreum* × *G. neglectum*	13_{II}		Denham (*10*).
	G. cernuum × *G. rudicum* (sic)	13_{II}		Do.
	G. sanguineum × *G. africanum*	13_{II}, occasionally 2_{I}	Highly fertile	Webber (*35*).
	G. herbaceum × *G. neglectum*	13_{II}	do	Do.
	G. herbaceum × *G. cernuum*	Mean: $0.20_{\text{I}}+12.9_{\text{II}}$	do	Webber (present article).
	G. herbaceum × *G. arboreum*	13_{II}; occasionally a few univalent	Fertile	Skovsted (*25*).
	G. herbaceum × *G. arboreum*, triploid	Mean: $2.5_{\text{I}}+5.7_{\text{II}}+6.0_{\text{III}}+1.1_{\text{IV}}+3.5_{\text{V}}+0.4_{\text{VI}}+0.05_{\text{VII}}$	Sterile	Do.
	G. sanguineum × *G. anomalum*	Mean: $2.36_{\text{I}}+11.82_{\text{II}}$	40 percent fertile	Webber ((*36*) and present article).
	G. arboreum × *G. anomalum*	Mean: $3.1_{\text{I}}+10.45_{\text{II}}+0.2_{\text{III}}+0.35_{\text{IV}}$		Skovsted (*29*).
	G. nanking × *G. anomalum*. 2 hybrids	Mean: $1.65_{\text{I}}+11.70_{\text{II}}+0.15_{\text{III}}+0.125_{\text{IV}}$		Do.
	G. africanum × *G. anomalum*, 2 hybrids	Mean: $2.5_{\text{I}}+11.75_{\text{II}}$		Do.
	(*G. herbaceum* × *G. neglectum*) × *G stocksii*	Mean: $24.60_{\text{I}}+0.70_{\text{II}}$	Sterile	Webber ((*36*) and present article)
	G. sanguineum × *G. stocksii*	Mean: $24.10_{\text{I}}+0.95_{\text{II}}$	Slightly fertile	Do.
	G. arboreum × *G. stocksii*. 5 hybrids	Mean: $13.48_{\text{I}}+6.25_{\text{II}}$		Skovsted (*29*).
	G. nanking × *G. stocksii*, 5 hybrids	Mean: $17.3_{\text{I}}+4.35_{\text{II}}$		Do.
	G. wightiana × *G. stocksii*. 5 hybrids	Mean. $19.6_{\text{I}}+3.2_{\text{II}}$		Do.
CATEGORY 2 Wild American ($n=13$) × wild American ($n=13$). (Group 3 × group 3).	*G. harknessii* × *G. armourianum*	13_{II}	Highly fertile	Webber (*35*).
	G. armourianum × *G thurberi*, 2 hybrids.	Mean: $0.60_{\text{I}}+12.70_{\text{II}}$	85 percent fertile	Webber ((*37*) and present article)
	G. thurberi × *Erioxylum aridum* (*G aridum*)	13_{II}	Sterile	Skovsted (*27, 29*).
	G. armourianum × *G. thurberi*	13_{II}; 1 pollen mother cell out of 45 = $2_{\text{I}}+12_{\text{II}}$.	Fertile	Skovsted (*29*)
	G. armourianum × *Erioxylum aridum* (*G. aridum*)	13_{II}		Do.
	G. davidsonii × *G. klotzschianum*	Mean: $0.10_{\text{I}}+12.95_{\text{II}}$	90 percent fertile	Webber (present article).
	G. harknessii × *G thurberi*	Mean: $0.80+12.60_{\text{II}}$	70 percent fertile	Do.

CATEGORY 3 Cultivated American ($n=26$) × cultivated American ($n=26$). (Group 4 × group 4).	*G. hirsutum* × *G. punctatum*	26_{II} occasionally 1, 2, or 3_{IV}	Highly fertile	Webber (*35*).
	G. hirsutum × *G. barbadense*	do	do	Do
	G. contextum × *G. schottii*	do	do	Do
	G. barbadense × *G. schottii*	do	do	Do
	G. barbadense × *G. punctatum*	do	do	Do
	G. barbadense × *G. tomentosum*	26_{II} in 5 pollen mother cells		Skovsted (*29*).
	G. hirsutum × *G. barbadense*	26_{II} in 5 pollen mother cells, $2_{I}+25_{II}$ in 1 pollen mother cell		Do.
	G. peruvianum × *G. darwinii*	Mean. $25.7_{I}+0.15_{IV}$	Highly fertile	Webber (present article).
	G. contextum × *G. hopei*	Mean $0.20_{I}+25.7_{II}+0.10_{IV}$	do	Do
	G. barbadense × *G. tomentosum*	26_{II}	do	Do.
	G. hirsutum × *G. tomentosum*, 2 hybrids	26_{II}	do	Do.
	G. purpurascens × *G. tomentosum*	26_{II}	do	Do
	G. sp., haploid	Few trisomes		Skovsted (*23*)
	G. barbadense, haploid	Mean $25.6_{I}+0.20_{II}$	Slightly fertile	Webber (*37*)
CATEGORY 4 *G. sturtii* ($n=13$)×wild American ($n=13$) (Group 1×group 3).	*G. sturtii* × *G. armourianum*	Mean $24.08_{I}+0.94_{II}$	Sterile	Webber (*37*)
	G. sturtii × *G. harknessii*	Mean $24.56_{I}+0.72_{II}$	Slightly fertile	Do.
	G. davidsonii × *G. sturtii*	Mean $23.94_{I}+1.03_{II}$	Sterile	Do.
	G. thurberi × *G. sturtii*	Mean $24.52_{I}+0.74_{II}$	do	Webber ((*35*) and present article).
	G. davidsonii × *G. sturtii*	Mean $14.95_{I}+5.33_{II}+0.1_{III}+0.03_{IV}$ 90 percent allosyndetic conjugation	do	Skovsted (*26*)
	G. sturtii × *G. armourianum*	Mean $8.45_{I}+8.20_{II}+0.25_{III}+0.1_{IV}$		Skovsted (*29*)
CATEGORY 5 Asiatic ($n=13$)×wild American ($n=13$). (Group 2×group 3).	*G. nanking* × *G. thurberi*	Mean $23.95_{I}+1.025_{II}$	Very slightly fertile	Webber ((*36*) and present article).
	G. arboreum × *G. thurberi*, 3 hybrids	Mean $10.08_{I}+7.58_{II}+0.2_{III}+0.04_{IV}$		Skovsted (*29*).
	G. nanking × *G. thurberi*, 2 hybrids	Mean $9.25_{I}+7.925_{II}+0.2_{III}+0.075_{IV}$		Do.
	G. anomalum × *Erioxylum aridum* (*G. aridum*)	Mean $21.15_{I}+2.35_{II}+0.05_{III}$	Sterile	Skovsted (*27, 29*)
	G. anomalum × *G. thurberi*	Mean $14.3_{I}+5.7_{II}+0.10_{III}$		Skovsted (*29*).
	G. anomalum × *G. davidsonii*	Mean $19.9_{I}+3.05_{II}$		Do.
	G. davidsonii × *G. anomalum*	Mean $25.6_{I}+0.2_{II}$	Sterile	Webber (present article).
CATEGORY 6 Asiatic ($n=13$)×*G. sturtii* ($n=13$) (Group 2×group 1)	*G. herbaceum* × *G. sturtii*	Mean $21.56_{I}+2.22_{II}$	do	Webber ((*36*) and present article).
	G. arboreum × *G. sturtii*, 2 hybrids	Mean $2.85_{I}+9.85_{II}+0.65_{III}+0.375_{IV}$	do	Skovsted (*27, 29*).

See footnotes at end of table.

TABLE 1.—*Summary of the meiotic chromosome conjugation and fertility of interspecific hybrids and variants in Gossypium*—Continued

Category of hybrids, whether within a group or between groups, and haploid chromosome numbers of the parent species	Hybrid or variant	Meiotic chromosome behavior [1]	Fertility [2]	Investigator and reference
CATEGORY 7 Cultivated American ($n=26$)×wild American ($n=13$). (Group 4× group 3).	*G.* sp.×*Erioxylum aridum* (*G. aridum*)	$13_{\text{II}}+13_{\text{I}}$	Slightly fertile	Skovsted (*24*).
	G. sp. × *G. harknessii*	$13_{\text{II}}+13_{\text{I}}$	do	Do.
	G. hirsutum × *G armourianum*	$13_{\text{II}}+13_{\text{I}}$	do	Webber (*34, 35*).
	G. contextum × *G. armourianum*	Mean. $13_{\text{I}}+12.72_{\text{II}}+0.14_{\text{IV}}$	do	Do.
	G. barbadense × *G. harknessii*	$13_{\text{II}}+13_{\text{I}}$	do	Do.
	G. hirsutum × *G. thurberi*, 2 hybrids	Mean: $13.06_{\text{I}}+12.70_{\text{II}}+0.18_{\text{III}}$	do	Webber ((*36*) and present article)
	G. barbadense × *Erioxylum aridum* (*G. aridum*).	Mean: $13.99_{\text{I}}+12.28_{\text{II}}+0.15_{\text{III}}$	Somewhat pollen-fertile	Skovsted (*29*).
	G. hirsutum × *Erioxylum aridum* (*G. aridum*).	Mean: $13.15_{\text{I}}+12.4_{\text{II}}+0.35_{\text{III}}$		Do.
	G. purpurascens × *Erioxylum aridum* (*G. aridum*).	Mean: $13.00_{\text{I}}+12.0_{\text{II}}+0.6_{\text{III}}+0.05_{\text{IV}}$		Do.
	G. barbadense × *G. armourianum*	Mean: $14.75_{\text{I}}+12.05_{\text{II}}+0.05_{\text{III}}$	Somewhat fertile	Do.
	G. hirsutum × *G. armourianum*, 2 hybrids.	Mean $12.675_{\text{I}}+12.75_{\text{II}}+0.275_{\text{III}}$		Do.
	G. purpurascens × *G. armourianum*, 2 hybrids.	Mean: $13.775_{\text{I}}+12.35_{\text{II}}+0.175_{\text{III}}$		Do.
	G. taitense × *G armourianum*	Mean $14.5_{\text{I}}+12.1_{\text{II}}+0.1_{\text{III}}$		Do.
	G. darwinii × *G. armourianum*	Mean. $13.2_{\text{I}}+12.00_{\text{II}}+0.6_{\text{III}}$		Do.
	G. barbadense × *G. thurberi*	Mean: $12.7_{\text{I}}+12.4_{\text{II}}+0.5_{\text{III}}$	Somewhat fertile	Skovsted (*27, 29*).
	G. hirsutum × *G. thurberi*	Mean: $13.5_{\text{I}}+12.45_{\text{II}}+0.2_{\text{III}}$	do	Do.
	G. taitense × *G. thurberi*	Mean: $13.4_{\text{I}}+12.05_{\text{II}}+0.5_{\text{III}}$		Skovsted (*29*).
CATEGORY 8 Cultivated American ($n=26$) × Asiatic ($n=13$). (Group 4 × group 2).	*G. hirsutum* × *G herbaceum*	$13_{\text{I}}+13_{\text{II}}$		Baranov (*4*).
	G. hirsutum × *G herbaceum*	$13_{\text{I}}+13_{\text{II}}$, nondisjunction of few bivalents.	Sterile	Nakatomi (*22*).
	G. barbadense × *G herbaceum*	do	do	Do.
	G hirsutum × *G. herbaceum*	$13_{\text{I}}+13_{\text{II}}$		Zhurbin (Emme (*12*)).
	Natural F_1	Variable conjugation, as high as $11_{\text{I}}+14_{\text{II}}$		Longley (*21*).
	G. barbadense × *G arboreum*	Mean $14.43_{\text{I}}+8.43_{\text{II}}+1.03_{\text{III}}+0.63_{\text{IV}}+0.25_{\text{V}}+0.15_{\text{VI}}$	Slightly fertile	Skovsted (*25*).
	G. nanking × *G hirsutum*	$13_{\text{I}}+13_{\text{II}}$, nondisjunction of few bivalents.	Self-sterile	Feng (*13*).
	G. hirsutum × *G. nanking*		Somewhat cross-fertile	
	Natural F_1	Mean. $13.71_{\text{I}}+11.94_{\text{II}}+0.35_{\text{IV}}$		Webber (*35*).
	G. hirsutum × *G cernuum*	Mean $13.22_{\text{I}}+12.74_{\text{II}}+0.1_{\text{III}}$ rarely non disjunction of 1 or 2_{II}	Somewhat fertile	Webber (present article).
	(*G. barbadense* × *G schottii*) × *G. anomalum*	Mean $18.12_{\text{I}}+10.32_{\text{II}}+0.08_{\text{III}}$	Sterile	Webber ((*36*) and present article).
	(*G contextum* × *G. hopi*) × *G. anomalum*.	Mean $17.81_{\text{I}}+10.48_{\text{II}}+0.11_{\text{III}}$	do	Do
	G. barbadense × *G anomalum*	Mean: $33.80_{\text{I}}+2.6_{\text{II}}$		Skovsted (*29*
	G barbadense × *G stocksii*	Mean $37.9_{\text{I}}+0.55_{\text{II}}$		Do.
	(*G arboreum* × *G herbaceum*) × (*G. hirsutum* × *G. barbadense*) $2n=52$	Mean $15.75_{\text{I}}+5.90_{\text{II}}+5.95_{\text{III}}+1.0_{\text{IV}}+0.4_{\text{V}}+0.1_{\text{VI}}$	Sterile	Skovsted (*25*).

CATEGORY 9 Cultivated American (n = 26) × *G. sturtii* (n=13). (Group 4 × group 1).	*G. barbadense* × *G. sturtii*	Mean: $37.18_{I}+0.91_{II}$	do	Webber (*34, 35*).
	G. barbadense × *G. sturtii*	Mean $32.5_{I}+3.10_{II}+0.1_{III}$	do	Skovsted (*29*).
	G. hirsutum × *G. sturtii*	Mean $20.95_{I}+7.6_{II}+0.95_{III}$	do	Do.
	G. purpurascens × *G. sturtii*	Mean $24.33_{I}+6.0_{II}+0.83_{III}+0.05_{IV}$	do	Do.
	G. darwinii × *G. sturtii*	Mean. $27.1_{I}+5.25_{II}+0.4_{III}+0.05_{IV}$	do	Do.
	G. hirsutum × *G. sturtii*, 3 hybrids	Mean $37.92_{I}+0.52_{II}$	do	Webber (present article).

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[1] The subscripts I, II, III, etc., are cytological symbols designating univalent, bivalent, trivalent, quadrivalent, pentavalent, hexavalent, and septavalent chromosomes.

[2] The percentage of fertility, indicated in the table and in the following paragraphs, was determined from the number of apparently good seeds in 3 or more selfed bolls and the number of apparently poor seeds and undeveloped ovules in the same bolls.

The writer has not observed secondary pairing in the wild American species.

Skovsted (*25*) reported that in cultivated American cottons 13 chromosomes are larger than the other 13; the larger ones being comparable in size to the chromosomes of Asiatic cottons and the smaller ones being equal in size to those of the wild American species. On the other hand, Arutjunova (*1*) found that while both *Gossypium hirsutum* and *G. herbaceum* have large and small chromosomes the chromosomes of *G. hirsutum* duplicate the small and large types of chromosomes in the Asiatic species. According to this investigator the chromosomes of *G. hirsutum* are in general somewhat smaller than those of Asiatic species. Webber (*35*) reported that the differences in chromosome size in cultivated American species are very slight and that there is a gradation of sizes rather than a sharp distinction between large and small chromosomes. Beal (*5*) found chromosome size to be of uniform gradation from very small to relatively large. Denham (*10*) and Davie (*8*) observed that two pairs of chromosomes in the cultivated American cottons were longer than the others. Secondarily paired bivalent chromosomes and quadrivalent chromosomes have been reported (*35*) in the cultivated American species. In haploid cultivated American species (table 1, category 3), Skovsted reported a few trisomes and Webber noted the occurrence of bivalent chromosomes.

INTERSPECIFIC HYBRIDS

HYBRIDS INVOLVING GOSSYPIUM STURTII (GROUP 1)

Cross-pollinations between *Gossypium sturtii* and species belonging to other groups of *Gossypium* are easily obtained. The F_1 plants are the most nearly sterile of any of the intergroup hybrids. In the past 3 years approximately 110 hybrids involving *G. sturtii* as one of the parents were grown. Although these plants flowered profusely during this period, they produced only two open-pollinated seeds. These seeds, of *G. sturtii* × *G. harknessii*, produced F_2 plants resembling the intermediate F_1.

The meiotic chromosome pairing in hybrids between cultivated American species and *G. sturtii* is shown in the following data on the first metaphase chromosome conjugation in pollen mother cells of three F_1 hybrids (W 81, W 82, and W 139) between *G. hirsutum* and *G. sturtii*.

Types of conjugation in 50 pollen mother cells:	*Number of pollen mother cells*
$37_I + 1_{II}$	8
$35_I + 2_{II}$	5
$33_I + 3_{II}$	3
39_I	34

Mean conjugation $= 37.92_I + 0.52_{II}$.

The foregoing tabulation shows that during meiosis there is a mean conjugation of 37.92 univalents and 0.52 bivalents in *Gossypium hirsutum* × *G. sturtii*. Although Skovsted (*29*) reported considerably more variable conjugation and the occurrence of a few trivalents and quadrivalents in similar hybrids, the mean conjugation in all observed hybrids between cultivated American species and *G. sturtii* (table 1, category 9) is similar.

The meiotic chromosome conjugation in Asiatic species × *G. sturtii* is shown in the following data on the first metaphase chromosome conjugation in pollen mother cells of *G. herbaceum* × *G. sturtii* F_1 (W 111).

Types of conjugation in 40 pollen mother cells:	*Number of pollen mother cells*
26_{I}	7
$24_{I}+1_{II}$	8
$22_{I}+2_{II}$	10
$20_{I}+3_{II}$	7
$18_{I}+4_{II}$	4
$16_{I}+5_{II}$	2
$14_{I}+6_{II}$	1
$12_{I}+7_{II}$	0
$10_{I}+8_{II}$	1

Mean conjugation—$21.56_{I}+2.22_{II}$.

In the foregoing tabulation it is shown that *Gossypium herbaceum* × *G. sturtii* F_1 forms 10 to 26 univalent and 0 to 8 bivalent chromosomes, with a mean conjugation of $21.56_{I}+2.22_{II}$ during meiosis. In similar hybrids Skovsted (*29*) observed a range of 0 to 14 univalents and 6 to 13 bivalents, with a mean of $2.85_{I}+9.85_{II}+0.65_{III}+0.375_{IV}$.

The writer (*35*) reported that there was no visible chromosome conjugation in *Gossypium thurberi* × *G. sturtii*. A further study of this hybrid, involving greater numbers of pollen mother cells, showed that it exhibited a limited amount of variable pairing. Following are the data on the first metaphase chromosome conjugation in pollen mother cells of *G. thurberi* × *G. sturtii* F_1 (W 58).

Types of conjugation in 50 pollen mother cells:	*Number of pollen mother cells*
26_{I}	29
$24_{I}+1_{II}$	11
$22_{I}+2_{II}$	6
$20_{I}+3_{II}$	2
$18_{I}+4_{II}$	2

Mean conjugation$=24.52_{I}+0.74_{II}$.

In analogous hybrids Webber found (table 1, category 4) the mean numbers of univalent chromosomes to range from 23.94 to 24.56 and those of bivalent chromosomes from 0.72 to 1.03. Skovsted (*26*, *29*) reported that the mean conjugation in similar hybrids was 8.45 to 14.95_{I}; 5.33 to 8.20_{II}; 0.10 to 0.25_{III}; and 0.03 to 0.10_{IV}. In a detailed study of *Gossypium davidsonii* × *G. sturtii*, Skovsted (*26*) was able to distinguish the parental chromosomes. He stated that the number of univalents is the same from both parents and that about 90 percent of the bivalents are formed by allosyndesis. The autosyndetic bivalents are formed in equal numbers by *G. davidsonii* and *G. sturtii*. Skovsted reported that a maximum of 10 allosyndetic and 2 autosyndetic bivalents were formed in this hybrid.

HYBRIDS INVOLVING ASIATIC SPECIES (GROUP 2)

Within the Asiatic group, excepting the two wild species (*Gossypium anomalum* and *G. stocksii*) interspecific hybrids are easily obtained and are highly fertile. The writer found that *G. anomalum* hybridizes fairly easily with *G. sanguineum* and produces a hybrid which is 40-percent fertile when selfed or backcrossed. *G. stocksii* is rather easily crossed with *G. sanguineum* and with *G. neglectum*. Six F_1 plants of *G. sanguineum* × *G. stocksii* produced only three open-pollinated seeds, and two F_1 plants of *G. neglectum* × *G. stocksii* failed to produce any seeds. Two hybrids between Asiatic species and wild American species, *G. davidsonii* × *G. anomalum* and *G. nanking* × *G. thurberi*, were obtained. The former hybrid is easily produced. Eight F_1 plants were grown but failed to produce a single seed. *G. nanking* × *G. thurberi* is one of the most difficult *Gossypium* hybrids to make. From nine F_1 plants[4] four open-pollinated seeds were collected. The writer obtained three hybrids between Asiatic species and cultivated American species. Although several natural hybrids between these groups have been reported, such hybrids are, nevertheless, among the most difficult to produce. A single F_1 plant of *G. hirsutum* × *G. cernuum* produced four open-pollinated seeds. On the other hand, one F_1 plant of the compound hybrid *G.* (*barbadense* × *schottii*) × *G. anomalum* and three F_1 plants of *G.* (*contextum* × *hopi*) × *G. anomalum* failed to form any seeds. Hybrids between Asiatic species and *G. sturtii* have been discussed in the preceding section.

The majority of plants in segregating generations of *Gossypium sanguineum* × *G. africanum* and *G. herbaceum* × *G. neglectum* differ considerably from each other and are highly fertile. Several F_3 plants of *G. sanguineum* × *G. africanum* resembled a different or third species, and one in particular resembled *G. nanking*. Five out of eight F_2 plants of *G. sanguineum* × *G. anomalum* failed to produce any seeds. The remaining three plants were, respectively, 5-, 12-, and 45-percent fertile. Phenotypically two of the plants resembled the *G. anomalum* parent, two the *G. sanguineum* parent, three the F_1 hybrid (intermediate), and one was similar to *G. africanum*. During germination experiments the three seeds from open-pollination of *G. sanguineum* × *G. stocksii* F_1 proved to be only empty seed coats. One of the four seeds from *G. nanking* × *G. thurberi* F_1 germinated. Since it gave rise to conjoined diploid twins, the formation of the seed probably involved sporophytic budding rather than fertilization (*37*). Three plants of *G. hirsutum* × *G. cernuum* F_2 were grown from seeds resulting from open pollination. They strongly resembled the *hirsutum* parent and were, respectively, 30-, 52-, and 65-percent fertile.

The meiotic chromosome pairing in hybrids between species within the Asiatic group is shown in the following data on the first metaphase chromosome conjugation in pollen mother cells of *Gossypium herbaceum* × *G. cernuum* F_1 (W 3).

	Number of pollen mother cells
Types of conjugation in 20 pollen mother cells:	
$2_I + 12_{II}$	2
13_{II}	18

Mean conjugation $= 0.20_I + 12.9_{II}$.

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[4] In cases where several plants have been grown of hybrids that are difficult to produce, the majority of them are cuttings from a single successful cross-pollination.

Following are the data on the meiotic chromosome conjugation in pollen mother cells of *G. sanguineum* × *G. anomalum* F_1 (W 27).

Types of conjugation in 50 pollen mother cells:	*Number of pollen mother cells*
13_{II}	26
$2_{I}+12_{II}$	8
$4_{I}+11_{II}$	5
$6_{I}+10_{II}$	6
$8_{I}+9_{II}$	3
$10_{I}+8_{II}$	1
$12_{I}+7_{II}$	1

Mean conjugation = $2.36_{I}+11.82_{II}$.

The meiotic chromosome pairing between species within the Asiatic group is likewise shown by the data given in table 2.

TABLE 2.—*Meiotic chromosome conjugation in pollen mother cells of the compound hybrid Gossypium (herbaceum × neglectum) × G. stocksii F_1 (W 98) and of G. sanguineum × G. stocksii F_1 (W 99)*

Types of conjugation in 40 pollen mother cells	Pollen mother cells in—	
	G. (herbaceum × neglectum) × G. stocksii	*G. sanguineum × G. stocksii*
	Number	*Number*
26_{I}	12	11
$24_{I}+1_{II}$	4	3
$22_{I}+2_{II}$	3	3
$20_{I}+3_{II}$	0	2
$18_{I}+4_{II}$	1	1
	Chromosomes	*Chromosomes*
Mean conjugation	$24_{I}+0.70_{II}$	$24.1_{I}+0.95_{II}$

Although *Gossypium herbaceum* × *G. cernuum* F_1 generally forms 13 bivalent chromosomes during meiosis, occasionally 2 univalents are present. The mean conjugation, 0.20 univalents and 12.9 bivalents, is comparable to that reported in analogous hybrids (table 1, category 1).

The data in table 2 and in the tabulation immediately preceding it show that hybrids within the Asiatic species group that involve either *Gossypium anomalum* or *G. stocksii* exhibit variable chromosome conjugation.

The data on *Gossypium sanguineum* × *G. anomalum* F_1 show that during meiosis this hybrid forms from 7 to 13 bivalent and from 0 to 12 univalent chromosomes, with a mean conjugation of 2.36 univalents and 11.82 bivalents. In analogous hybrids, Skovsted also found considerable pairing (table 1, category 1). Although chromosome conjugation in *G.* (*herbaceum* × *neglectum*) × *G. stocksii* and *G. sanguineum* × *G. stocksii* is likewise variable, the number of pairs is considerably less. In these hybrids, from 0 to 4 bivalent and from 18 to 26 univalent chromosomes are formed. The mean numbers of bivalents observed by the writer (table 2) were 0.7 and 0.95, while Skovsted reported (table 1, category 1) mean bivalent numbers of 3.2, 4.35, and 6.25 in similar hybrids.

The meiotic chromosome pairing in hybrids between Asiatic species and wild American species is shown in table 3.

TABLE 3.—*Meiotic chromosome conjugation in pollen mother cells of Gossypium davidsonii × G. anomalum F_1 (W 94) and G. nanking × G. thurberi F_1 (W 79)*

Types of conjugation in 80 pollen mother cells	Pollen mother cells in—	
	G. davidsonii × G. anomalum	*G. nanking × G. thurberi*
	Number	*Number*
26_{I}	34	18
$24_{I}+1_{II}$	4	9
$22_{I}+2_{II}$	2	8
$20_{I}+3_{II}$	0	4
$18_{I}+4_{II}$	0	1
	Chromosomes	*Chromosomes*
Mean conjugation	$25.6_{I}+0.2_{II}$	$23.95_{I}+1.025_{II}$

Table 3 shows that the number of bivalent chromosomes during the heterotypic division of *Gossypium davidsonii* × *G. anomalum* F_1 and *G. nanking* × *G. thurberi* F_1, respectively, ranges from 0 to 2, with a mean of 0.2, and from 0 to 4, with a mean of 1.025. In similar hybrids (table 1, category 5) Skovsted found considerably more meiotic pairing, with the occasional formation of trivalent and quadrivalent chromosomes.

The meiotic chromosome pairing in hybrids between Asiatic species and cultivated American species is summarized in the following data on the meiotic chromosome conjugation in pollen mother cells of *G. hirsutum* × *G. cernuum* F_1 (W 21) and in table 4.

Types of conjugation in 50 pollen mother cells:	*Number of pollen mother cells*
$17_{I}+11_{II}$	2
$15_{I}+12_{II}$	4
$13_{I}+13_{II}$	41
$12_{I}+12_{II}+1_{III}$	1
$11_{I}+11_{II}+2_{III}$	2

Mean conjugation $=13.22_{I}+12.74_{II}+0.1_{III}$.

TABLE 4.—*Meiotic chromosome conjugation in pollen mother cells of the compound hybrids Gossypium (contextum × hopi) × G. anomalum* F_1 (W 55) *and G. (barbadense × schottii) × G. anomalum* F_1 (W 1)

Types of conjugation in 150 pollen mother cells	Pollen mother cells in—		Types of conjugation in 150 pollen mother cells	Pollen mother cells in—	
	G. (contextum × hopi) × G. anomalum	*G. (barbadense × schottii) × G. anomalum*		*G. (contextum × hopi) × G. anomalum*	*G. (barbadense × schottii) × G. anomalum*
	Number	*Number*		*Number*	*Number*
$27_{I}+6_{II}$	3	1	$12_{I}+12_{II}+1_{III}$	3	1
$25_{I}+7_{II}$	4	2	$11_{I}+11_{II}+2_{III}$	1	0
$23_{I}+8_{II}$	9	5	$10_{I}+10_{II}+3_{III}$	2	1
$21_{I}+9_{II}$	10	6			
$19_{I}+10_{II}$	17	12		*Chromosomes*	*Chromosomes*
$17_{I}+11_{II}$	21	9	Mean conjugation	$17.81_{I}+10.48_{II}+0.11_{III}$	$18.12_{I}+10.32_{II}+0.08_{III}$
$15_{I}+12_{II}$	18	8			
$13_{I}+13_{II}$	12	5			

The data on *Gossypium hirsutum* × *G. cernuum* F_1 show that this hybrid generally forms 13 bivalent and 13 univalent chromosomes during meiosis. Occasionally, however, pairing is slightly less and rarely trivalent chromosomes are formed. The pairing in this hybrid is comparable to that reported for all analogous hybrids except those observed by Skovsted (table 1, category 8). Although Skovsted agrees regarding the number of univalents, he found that quadrivalent, pentavalent, and hexavalent chromosomes are formed occasionally.

Meiotic chromosome conjugation in the compound hybrids *Gossypium* (*contextum* × *hopi*) × *G. anomalum* F_1 and *G.* (*barbadense*×*schottii*) × *G. anomalum* F_1 is the most variable observed by the writer among interspecific hybrids in *Gossypium*. Table 4 shows that conjugation in these hybrids ranges from 6 to 13 bivalents, from 10 to 27 univalents, and from 0 to 3 trivalents, with mean conjugations of $17.81_{I}+10.48_{II}+0.11_{III}$ and $18.12_{I}+10.32_{II}+0.08_{III}$. In *G. barbadense*×*G. anomalum* F_1 Skovsted reported a mean conjugation of $33.80_{I}+2.6_{II}$.

Skovsted (table 1, category 8) found slightly less pairing in *G. barbadense* × *G. stocksii* F_1.

The meiotic chromosome conjugation of hybrids between Asiatic species and *G. sturtii* has been discussed in the preceding section.

All plants examined of *G. sanguineum* × *G. africanum* F_2 and F_3 exhibited meiotic chromosome conjugation similar to that of their F_1 parent (table 1, category 1). Only three plants of *G. sanguineum* × *G. anomalum* F_2 were examined. These plants also resembled their F_1 parent in having variable pairing. Although the F_2 plants of *G. hirsutum* × *G. cernuum* (table 1, category 8) occasionally formed trivalent and quadrivalent chromosomes and rarely pentavalents, they generally had $4_{I}+22_{II}$, $1_{I}+25_{II}$, and $1_{I}+26_{II}$.

HYBRIDS INVOLVING WILD AMERICAN SPECIES (GROUP 3)

Hybrids between *Gossypium armourianum* and *G. harknessii* and between *G. davidsonii* and *G. klotzschianum* are easily made. The F_1's are highly fertile when selfed or backcrossed. *G. thurberi* is rather easily crossed with *G. armourianum* and with *G. harknessii*. *G. armourianum* × *G. thurberi* F_1 is approximately 85-percent self-fertile, and *G. harknessii* × *G. thurberi* F_1 is about 70-percent self-fertile. Both hybrids are highly fertile when pollinated by pollen from either parent. On the other hand, numerous cross-pollinations between *G. davidsonii* or *G. klotzschianum* and *G. armourianum*, *G. harknessii*, or *G. thurberi* produced only empty seed coats. However, recent experiments indicate that *G. davidsonii* may be combined with *G. armourianum* or with *G. harknessii* in triple hybrids involving *G. thurberi*.

Hybrids of *Gossypium armourianum*, *G. harknessii*, or *G. thurberi* with cultivated American species are somewhat difficult to produce. Twenty-two plants of *G. hirsutum* × *G. armourianum* F_1 produced 4 seeds from self-pollination and 15 seeds from open pollination; 15 plants of *G. barbadense* × *G. harknessii* F_1 produced 4 seeds from self-pollination and 30 seeds from open pollination; and 5 plants of *G. hirsutum* × *G. thurberi* F_1 produced 2 seeds from self-pollination and 16 seeds from open pollination. These hybrids are slightly more fertile when backcrossed with their cultivated American parent than when selfed.

Mature plants of hybrids of *Gossypium davidsonii* or *G. klotzschianum* with cultivated American species were not obtained. Numerous attempts to cross *G. davidsonii* or *G. klotzschianum* with *G. hirsutum* or *G. barbadense* failed completely or produced only empty seed coats. Although hybrids between *G. purpurascens* and *G. klotzschia-*

num are rather easily produced, all die in the cotyledon stage of development.

Interspecific hybrids between *Gossypium sturtii* and the wild American species, and between Asiatic species and wild American species have been discussed in preceding sections of this paper.

Only a few plants were grown of *Gossypium armourianum* × *G. harknessii* F_2 and *G. klotzschianum* × *G. davidsonii* F_2. In each case these plants resembled the intermediate F_1 plants and were fairly self-fertile. Of 59 plants of *G. armourianum* × *G. thurberi* F_2, 31 produced flowers. Of these, 19 were sterile, 7 were slightly fertile, 4 were moderately fertile, and 1 was highly fertile. Phenotypically the plants were highly variable, ranging from plants similar to *G. armourianum* to plants resembling *G. thurberi*. *G. armourianum* × *G. thurberi* F_1, when backcrossed to *G. thurberi*, is highly fertile and very closely resembles *G. thurberi*. On the other hand, when backcrossed to *G. armourianum*, it is only moderately fertile, although the F_1 parent selected resembled *G. armourianum* very closely. The F_2 of *G. harknessii* × *G. thurberi* was not grown.

Four plants from self-pollination and three plants from open pollination of *Gossypium hirsutum* × *G. armourianum* F_2 were grown. All except one of the plants resembled the *G. hirsutum* parent. One of the F_2 plants from seed resulting from open pollination closely resembled the intermediate condition characteristic of F_1 plants Plants resulting from self-pollination were, respectively, about 0-, 55-, 60-, and 85-percent fertile. Those resulting from open pollination were approximately 0-, 35-, and 70-percent fertile. Four plants from self-pollination and four plants from open pollination of *G. barbadense* × *G. harknessii* F_2 were grown. One of the plants from self-pollination resembled *G. barbadense* × *G. hirsutum* F_1, whereas the other three resembled the *G. barbadense* parent. They were, respectively, about 50-, 75-, 75-, and 80-percent fertile. Of the plants resulting from open pollination of the F_2, two resembled *G. barbadense*, one resembled *G. hirsutum*, and one resembled *G. barbadense* × *G. hirsutum* F_1. They were, respectively, about 85-, 0-, 80-, and 50-percent fertile. In the inbred lineage 17 F_3 plants were grown from an F_2 plant which was 50-percent fertile and which resembled phenotypically *G. barbadense* × *G. hirsutum* F_1. The phenotype of the F_3 plants varied considerably. In general, 10 of these plants resembled the *G. barbadense* parent, 2 resembled *G. hirsutum*, and 1 each resembled *G. peruvianum*, *G. darwinii*, *G. barbadense* × *G. darwinii* F_1, *G. barbadense* × *G. brasiliense* F_1, and *G. barbadense* × *G. hirsutum* F_1. The plants showed such floral abnormalities as stigmatoid anthers and petals, antheroid stigmas and petals, imbricate petals, and cleistogamous flowers. Five of the F_3 plants were sterile, 10 ranged from approximately 15- to 80-percent fertile, and 2 did not flower.

Only two F_1 plants of the compound hybrid *Gossypium* (*barbadense* × *harknessii*) × *G. barbadense* were grown. These plants resembled *G. barbadense* and were moderately fertile.

No F_2 plants of *G. hirsutum* × *G. thurberi* were grown.

Table 5 summarizes the meiotic chromosome conjugation of interspecific hybrids within the wild American group of species.

TABLE 5.—*Meiotic chromosome conjugation in pollen mother cells of Gossypium davidsonii × G. klotzschianum F_1 (W 10), G. armourianum × G. thurberi F_1 (W 8 and W 9), and G. harknessii × G. thurberi F_1 (W 96)*

Interspecific hybrid	Pollen mother cells showing indicated type of conjugation Total, 60 pollen mother cells			Mean conjugation
	4_I+11_{II}	2_I+12_{II}	13_{II}	
	Number	*Number*	*Number*	
G. davidsonii × G. klotzschianum	------	1	19	$0.10_I+12.95_{II}$.
G. armourianum × G. thurberi	2	2	16	$.60_I+12.70_{II}$
G. harknessii × G. thurberi	3	2	15	$.80_I+12.60_{II}$

Table 5 shows that hybrids within the wild American group of species generally exhibit 13 bivalent chromosomes, though occasionally as many as 4 univalent chromosomes are present. Similar chromosome pairing has been reported previously in analogous hybrids among wild American species (table 1, category 2). Such pairing is also comparable to that reported by Skovsted (table 1, category 2) in hybrids of *Gossypium thurberi* or *G. armourianum* with *Erioxylum aridum* (*G. aridum*).

The meiotic chromosome conjugation in pollen mother cells of *Gossypium hirsutum* × *G. thurberi* F_1 (W 37 and W 44) is shown in the following tabulation:

Types of conjugation in 50 pollen mother cells:	*Number of pollen mother cells*
17_I+11_{II}	1
15_I+12_{II}	3
13_I+13_{II}	39
$12_I+12_{II}+1_{III}$	4
$14_I+11_{II}+1_{III}$	1
$11_I+11_{II}+2_{III}$	2

Mean conjugation—$13.06_I+12.70_{II}+0.18_{III}$.

Although *Gossypium hirsutum* × *G. thurberi* F_1 generally forms 13 bivalent and 13 univalent chromosomes, it occasionally forms fewer bivalents and rarely forms trivalents. Similar chromosome pairing in different types of wild American and cultivated American interspecific hybrids has previously been reported (table 1, category 7).

The meiotic chromosome behavior in hybrids between wild American species and *Gossypium sturtii* and between wild American species and Asiatic species has been described earlier in this paper.

Several plants each of *Gossypium armourianum* × *G. harknessii* F_2 and *G. klotzschianum* × *G. davidsonii* F_2 were examined. In each case, their meiotic behavior was similar to that of the F_1. Fertile and sterile plants of *G. armourianum* × *G. thurberi* F_2 also exhibited meiotic chromosome conjugation similar to that of the F_1.

Although F_2 plants from seeds resulting from self-pollination of *Gossypium hirsutum* × *G. armourianum* F_1 occasionally exhibited trivalents, the common types of conjugation in these plants were 4_I+22_{II}, 2_I+24_{II}, 1_I+25_{II}, and 13_I+26_{II},[5] whereas two plants from seeds resulting from open-pollination had $12_{I}+14_{II}$ and $4_{I}+26_{II}$. F_2 plants from self-pollination of *G. barbadense* × *G. harknessii* exhibited $2_{I}+24_{II}$, 26_{II}(?), $1_{I}+25_{II}$, and 26_{II}. Only two plants of *G. barbadense* × *G. harknessii* F_3 ($2_{I}+24_{II}$) were examined. One of these plants was 60-percent fertile, of *G. barbadense* type, and had 26_{II}. The other plant was sterile, resembled *G. barbadense* × *G. darwinii* F_1, and had $1_{I}+25_{II}$.

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[5] This plant, of pentaploid nature, probably arose from the union of 39- and 26-chromosome gametes, the 39-chromosome gamete having arisen from a suspended mitosis and the 26-chromosome gamete from a 26<>13 first anaphase distribution. It is interesting that, with the exception of one plant with 4_I+26_{II} and one plant with 1_I+26_{II}, all F_2 and F_3 plants total 26 chromosome units. It is probable that at least one of the gametes concerned in the formation of a zygote must have 26 chromosomes.

HYBRIDS INVOLVING CULTIVATED AMERICAN SPECIES (GROUP 4)

Hybrids between cultivated American species and the three groups of 13-chromosome species have been discussed in the preceding pages (table 1, categories 7, 8, 9). Interspecific crosses within the cultivated American group are the most successful of any interspecific *Gossypium* crosses. The F_1 plants are highly fertile.

The meiotic chromosome conjugation of six interspecific hybrids within the cultivated American group of species is summarized in table 6.

TABLE 6.—*Meiotic chromosome conjugation in pollen mother cells of six interspecific hybrids (F_1) within the cultivated American group of Gossypium species*

Interspecific hybrid	Pollen mother cells showing indicated type of conjugation (total, 100 pollen mother cells)				Mean conjugation
	$2_{I}+25_{II}$	26_{II}	$24_{II}+1_{IV}$	$22_{II}+2_{IV}$	
	Number	*Number*	*Number*	*Number*	
G. peruvianum×*G. darwinii* (W 78)	--------	18	1	1	$25.7_{II}+0.15_{IV}$.
G. contextum×*G. hopi* (W 26)	2	16	2	--------	$0.20_{I}+25.7_{II}+0.10_{IV}$
G. barbadense×*G. tomentosum* (W 28)	--------	20	--------	--------	26_{II}
G. hirsutum×*G. tomentosum* (W 29 and W 80)	--------	20	--------	--------	26_{II}
G. purpurascens×*G. tomentosum* (W 95)	--------	20	--------	--------	26_{II}

Although interspecific hybrids within the cultivated American group generally exhibit normal meiotic chromosome conjugation, they occasionally form several univalent and quadrivalent chromosomes (table 6). Behavior similar to that shown in table 6 has previously been reported in other hybrids between cultivated American species (table 1, category 3).

DISCUSSION

GENETICAL NATURE OF CHROMOSOME COMPLEMENTS

The fact that hybrids between the wild American species and *Gossypium sturtii* exhibit very little chromosome conjugation indicates that there is very little, if any, homology within the haploid chromosome sets of these groups. Hence, the evidence that either of these species is of polyploid origin is insufficient. Likewise, the lack of secondary association in Asiatic species and the limited conjugation in hybrids between cultivated Asiatic species and *G. stocksii* indicate that if homology exists within the haploid complements of Asiatic species it is very limited. On the other hand, the occurrence of highly polyvalent chromosomes in triploid *G. herbaceum* × *G. arboreum* F_1 (table 1, category 1) and of similar somatic chromosome pairs in *G. herbaceum* (*1*) supports the assumption that the cultivated Asiatic species are of polyploid nature.

The occurrence of bivalents in haploid *G. barbadense* and the presence of quadrivalents and secondary bivalent association in diploid cultivated American species indicate some homology within the haploid chromosome complement of the cultivated American cottons. Further proof of this homology is found in the formation of trivalents in hybrids between cultivated American species and the 13-chromosome species.

INTERSPECIFIC HYBRIDS AND PHYLOGENY

Since *Gossypium sturtii* gives viable hybrids with species of all other groups of *Gossypium*, it is evident that it is more or less related to each group; but the high degree of sterility and the very limited chromosome conjugation in hybrids involving *G. sturtii* suggest that these relationships are rather distant.

In the case of *Gossypium sturtii* × Asiatic species, and possibly *G. davidsonii* × *G. sturtii*, the findings of Skovsted (*26*) and Webber (*35*) are so unlike as to suggest very different conceptions of the relationships. In *G. arboreum* × *G. sturtii* Skovsted (*27*, *29*) found a degree of conjugation adequate to justify the inclusion of *G. sturtii* in the Asiatic group. As indicated above, the sterility and slight amount of conjugation in an analogous hybrid observed by Webber fail to denote close relationship between *G. sturtii* and Asiatic species. Furthermore, hybrids involving Asiatic species other than *G. stocksii* exhibit chromosome conjugation quite different from that of hybrids of which *G. sturtii* was a parent. Skovsted's observation of 10, out of a possible 13, allosyndetic bivalents in *G. davidsonii* × *G. sturtii* suggests, in the writer's opinion, a fairly close relationship between these species. For reasons similar to those given in the case of *G. sturtii* × Asiatic species, Webber's findings indicate, on the contrary, that *G. davidsonii* and *G. sturtii* are not closely related.

The high fertility and complete meiotic chromosome conjugation of hybrids among cultivated Asiatic species denote that these species are closely interrelated. The similarity of F_2 and F_3 segregates to species other than the parents of the F_1 suggests that the majority of the numerous so-called species of the Asiatic group arose through hybridization. Such derivation supports recent taxonomic conclusions that many of the cultivated Asiatic cottons described as species are not entitled to this rank.

Since hybrids between *Gossypium anomalum* and the cultivated Asiatic species exhibit a moderately high degree of fertility and of chromosome conjugation, it is apparent that the parental species are somewhat closely related. On the other hand, the sterility and lack of chromosome conjugation in the compound hybrid *G.* (*herbaceum* × *neglectum*) × *G. stocksii* show that *G. stocksii* is distantly related to the cultivated Asiatic species. It therefore follows that *G. anomalum* and *G. stocksii* are not closely related, since the chromosomes of *G. anomalum* are partly homologous with those of the cultivated Asiatic species while the chromosomes of *G. stocksii* are not.

That the cultivated Asiatic species and *Gossypium anomalum* (group 2) are not closely related to the wild American species (group 3) is shown by the lack of fertility and of chromosome pairing in hybrids between these groups. No hybrids between *G. stocksii* and the wild American species have yet been produced. In the following paragraphs it is shown that the chromosomes of the majority of wild

American species are cytologically homologous with 13 chromosomes of the cultivated American species. On the other hand, data in table 1, category 8, indicate that the chromosomes of *G. stocksii* lack such affinity for those of the cultivated American species. Hence, the chromosomes of *G. stocksii* and those of the wild American species are undoubtedly nonhomologous, and *G. stocksii* is apparently very distantly related to the wild American species.

The occurrence of complete conjugation between the 13 haploid chromosomes of the cultivated Asiatic species and 13 chromosomes of the cultivated American species[6] indicates a rather close relationship between these groups of species. Similar reasons indicate that *Gossypium anomalum* is somewhat closely related to the cultivated American species, while the lack of chromosome pairing in cultivated American species × *G. stocksii* denotes that they are distantly related.

Gossypium armourianum and *G. harknessii* are undoubtedly very closely related, since they produce highly fertile hybrids that exhibit normal chromosome conjugation. Likewise, all cytogenetic data indicate a close relationship between *G. davidsonii* and *G. klotzschianum*. On the other hand, the probability that *G. armourianum* and *G. harknessii* are phylogenetically distinct from *G. davidsonii* and *G. klotzschianum* is indicated by the failure to obtain hybrids between *G. armourianum* (or *G. harknessii*) and *G. davidsonii* (or *G. klotzschianum*), and by the fact that *G. armourianum* and *G. harknessii* hybridize readily with the cultivated American species whereas *G. davidsonii* and *G. klotzschianum* do not. The latter fact is noteworthy because morphologically *G. davidsonii* and *G. klotzschianum* show greater resemblance to the cultivated American cottons than do *G. harknessii* and *G. armourianum*.

Webber (*36*) and Skovsted (*29*) have found *Gossypium thurberi* and *Erioxylum aridum* to hybridize rather freely with both *G. armourianum* and *G. harknessii*, producing fairly fertile hybrids, which exhibit normal chromosome conjugation. Skovsted (*29*) found that *G. thurberi* × *E. aridum* exhibited complete chromosome pairing. The foregoing data indicate a close relationship between *G. thurberi*, *E. aridum*,[7] *G. armourianum*, and *G. harknessii*. On the other hand, the fact that combination between *G. davidsonii* and *G. armourianum* has been obtained only in triple hybrids involving *G. thurberi* suggests that *G. thurberi* may be an intermediate species. A closer relationship between *G. armourianum* and *G. harknessii* than between these species and *G. thurberi* or *E. aridum* is also indicated by histological data. Webber (*32*) has pointed out that while the mesophyll of *G. armourianum* is similar to that of *G. harknessii*, the mesophyll of *G. thurberi* and of *E. aridum* resembles that of *G. davidsonii* and *G. klotzschianum*. That the relationship of *E. aridum* may not be as close as that of *G. thurberi* to *G. harknessii* and *G. armourianum* is suggested by Webber's (*32*) report that lysigenous cavities such as occur in the pith of *Erioxylum* are not characteristic of the pith of any of these species of *Gossypium*.

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[6] That this pairing is between chromosomes of the Asiatic and the American species, rather than among chromosomes within the haploid set of the American species, is shown by the lack of conjugation in haploid *Gossypium barbadense* and in certain hybrids such as *G. hirsutum* × *G. sturtii*.

[7] On the basis of these results, Skovsted (*28*) proposed the transfer of *Erioxylum aridum* Rose and Standley to the genus *Gossypium*, as *G. aridum* Skovsted.

In preceding sections cytological indications are given of relationship between the wild American species, on the one hand, and *Gossypium sturtii* and certain species of the Asiatic group, on the other. Since, however, no hybrids have been obtained between *G. davidsonii* or *G. klotzschianum* and *G. stocksii*, or between *G. davidsonii* or *G. klotzschianum* and the other wild American species, there are no available cytological data indicative of the relationships between *G. davidsonii* or *G. klotzschianum* and *G. stocksii*. On the other hand, since the chromosomes of *G. davidsonii* and *G. anomalum* are nonhomologous, whereas the chromosomes of *G. anomalum* are partly homologous with 13 of those of the cultivated American species, it would be inferred that the chromosomes of *G. davidsonii* and those of *G. anomalum* are both nonhomologous with the same set of chromosomes in the cultivated American species. In this case, however, the evidence is not conclusive, since it is very indirect, and based on limited homology between the chromosomes of *G. anomalum* and those of the cultivated American species.

As in the case of the Asiatic species, the occurrence of complete conjugation between the 13 haploid chromosomes of *Gossypium armourianum*, *G. harknessii*, or *G. thurberi* and 13 of the 26 chromosomes of the cultivated American species indicates a fairly close relationship between these three wild American species and the cultivated American species. However, since the chromosomes of the wild American species and those of the Asiatic species are nonhomologous, it may be inferred that the 13 chromosomes of the wild American species and the 13 chromosomes of Asiatic species pair with different sets of 13 chromosomes in cultivated American cottons.

The normal chromosome conjugation and high fertility of interspecific hybrids among cultivated American species (table 1, category 3) denote a close relationship among the species of this group.

It is apparent that the majority of segregates from combinations between species having 13 and 26 chromosomes, respectively, revert to forms having 26 bivalents. Whether these forms will maintain their distinctive characters or revert to the characters of the parent having 26 pairs has not been determined. It is, however, possible that some of the numerous cultivated American forms may have been derived from hybrids between parents having 13 and 26 chromosomes, respectively.

PHYLOGENETIC CONCEPTIONS

Within the haploid complements of the 13-chromosome species of *Gossypium*, Skovsted (*23*, *26*, *29*) observed autosyndetic pairing in the Asiatic species and *G. sturtii*, and autosyndetic and secondary pairing in the wild American species. He offered two alternative explanations as to the origin of these species: That they are (1) derived from a triple cross of two different but closely allied seven-chromosome species with the same six-chromosome species or (2) from the doubling of two similar six-chromosome sets, and the triple appearance of one chromosome. Davie (*8*) observed that one pair of chromosomes in the Asiatic cottons is longer than the others and that the species of this group exhibited considerable secondary pairing. This led him to conclude that the species with 13 pairs of chromosomes probably arose by end-to-end fusion of certain chromosomes in an allotetraploid with 28 chromosomes.

The writer has found no conclusive evidence that homology exists within the haploid chromosome sets of any of the 13-chromosome species. The limited pairing that occurs in hybrids between species of the different groups characterized by 13 pairs of chromosomes may be between the two species sets of 13 chromosomes rather than within one or both sets. Although Denham (*10*) and Davie (*8*) found that one pair of chromosomes in the Asiatic species is longer than the others, this observation is contrary to the findings of Skovsted (*25*), Arutjunova (*1*), and Webber (*33*). Davie's report that secondary association occurs in Asiatic species was unconfirmed by the observations of Skovsted (*25*) and Webber (*33*), while Skovsted's report (*29*) that secondary pairing takes place in the wild American species is contrary to the findings of Webber (*33*). There is also disagreement between Webber (*35*) and Skovsted (*26*) as to the extent and type of pairing in *Gossypium davidsonii* × *G. sturtii*. Hence, although it would seem that the 13-chromosome species of the genus *Gossypium* may have originated from another genus having a lower basal chromosome number, the evidence as to how they originated or what was the ancestral form is still too meager and contradictory to form the basis for definite conclusions.

It is highly probable that the cultivated American species are polyploids of species having 13 pairs of chromosomes. However, it is still questionable whether they are autopolyploids or allopolyploids. If they are allopolyploids there is no indication as to how close was the relationship between the 13-chromosome species that contributed to their ancestry. Dr. Edgar Anderson has suggested [8] that the cytology of Malvaceae leads to the supposition that many species may actually be autopolyploids although all species that have been investigated behave as allopolyploids. Darlington (*7*) has explained such behavior of autopolyploids as due to low chiasma frequency. In *Gossypium* it has been shown (*29, 33*) that the chiasma frequency per bivalent is relatively high within a species and in hybrids between species of the same group but is considerably lower in hybrids between species of different groups. Hence, the frequency of chiasmata in the cultivated American cottons suggests that these species are true allopolyploids rather than autopolyploids behaving as allopolyploids.

The limited chromosome conjugation in haploid *Gossypium barbadense* and in hybrids involving the cultivated American species and 13-chromosome species also supports the assumption that the cultivated American species are allopolyploids. On the other hand, the formation of polyvalents in triploid Asiatic cotton led Skovsted (*23*) to state that conjugation in the triploid must be due to the triploid offering possibilities which, although present in the diploid, do not normally become realized there. "The pairing in the diploid shows only a 'phenotypic conjugation', while the triploid exhibits more of the 'genotypic conjugation' " * * *. (*23*, p. 243). That the complete amount of chromosome homology is not cytologically expressed in diploid cottons is also indicated by the occurrence of trisomes in haploid cultivated American species (*23*), and by the occurrence of more polyvalent chromosomes and of higher degrees of polyvalency in intergroup *Gossypium* hybrids than within the species.

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[8] Letter to the writer dated January 12, 1937.

Skovsted (*25*) concluded that the cultivated American cottons are allopolyploids whose origin may be ascribed to the doubling of the chromosomes in a hybrid between an Asiatic and a wild American species. This hypothesis was suggested by his observation that half of the chromosomes of the cultivated American species were relatively large like those of the Asiatic species and that half were smaller like those of the wild American species. Later Skovsted (*29*), after calling attention to the fact that his hypothesis had been criticized chiefly because it involved an Asiatic species in the origin of the cultivated American species, supported his hypothesis with the following evidence: (1) The haploid complement of the cultivated American species is composed of two nonhomologous sets of 13 chromosomes, one of which is homologous with the haploid complement of Asiatic species and the other homologous with the haploid complement of the wild American species (*24, 25, 33, 34*); (2) the chromosomes of the wild American species apparently conjugate with the set of smaller chromosomes in cultivated American cottons (*29*); (3) the "*R*" gene transferred from *Gossypium arboreum* to *G. hirsutum* acts as a member of the cultivated American cotton series of anthocyanin allelomorphs (*18*); and (4) cytological homology exists throughout the group of New World cottons despite their wide distribution in the Americas and Polynesia.

The genetical behavior of the cultivated American cottons caused Longley (*21*) to infer that they are true tetraploids. Davie (*8*) considered the cultivated American cottons to be allopolyploids and suggested *Gossypium armourianum*, *G. harknessii*, *G. davidsonii*, or *G. klotzschianum* as possible parents. Arutjunova (*1*) considered the species of *Gossypium* with 26 pairs of chromosomes to be amphidiploids, each derived from two very closely related species having morphologically similar sets of chromosomes. Although Gates (*14*) granted that the cultivated American species may have arisen in post-Columbian time from hybrids between Asiatic and wild American species, he favored the hypothesis that they originated from wild American species and suggested that an unknown wild American species may have been involved. The latter hypothesis is supported, in the writer's view, by the following evidence: The presence of two sets of morphologically similar chromosomes in cultivated American species; the formation of quadrivalent chromosomes in diploid and of bivalents in haploid cultivated American cottons; the relatively close geographical proximity, yet isolation from one another, of several of the species of wild American cottons; and the fact that, aside from the absence of long lint hairs on the seeds, the wild American cottons possess all the important phenotypic characters that are found in the cultivated American cottons. In the latter connection it should be noted that several F_2 plants of *G. armourianum* × *G. thurberi* greatly resemble *G. hirsutum* and that certain F_2 and F_3 plants of *G. barbadense* × *G. harknessii* resemble cultivated American species other than *G. barbadense*.

Skovsted (*29*) called attention to homology between the chromosomes of *Gossypium armourianum*, *G. harknessii*, and *G. thurberi*, and the absence of homology between the chromosomes of these species and those of *G. davidsonii* and *G. klotzschianum*. The failure of *G. davidsonii* and *G. klotzschianum* to hybridize with *G. armourianum*, *G. harknessii*, or *G. thurberi*, and the production of nonviable hybrids between *G. davidsonii* or *G. klotzschianum* and the cultivated American species, led Skovsted to conclude that allopolyploids could have originated only in hybrids between a species of the Asiatic group and a wild American species of the *G. armourianum*, *G. harknessii*, and *G. thurberi* subgroup. In this connection it should be noted that wild American × Asiatic hybrids are extremely difficult to make. Furthermore, as Skovsted stated, the failure of *G. davidsonii* or *G. klotzschianum* to hybridize with other wild American species may be due to a simple genetic difference. Skovsted (*29*) also reported a close relationship between *G. sturtii* and the Asiatic species, and observed in *G. davidsonii* × *G. sturtii* as many as 10 allosyndetic bivalents out of a possible 13. The writer believes that pairing to this extent indicates a rather close relatonship between these two species.

Skovsted also called attention to the fact that if it should be proved that the cultivated American species were derived solely from wild American species the homology between the chromosomes of the Asiatic species and 13 chromosomes of the cultivated American cottons remains to be accounted for. This is true, unless the existence of such homology should be disproved. The possibility of the cultivated American species being autopolyploids that generally behave as allopolyploids and exhibit autopolyploid behavior only under rare or specific conditions should not be overlooked. It is also possible that *Gossypium davidsonii* or *G. klotzschianum* or some of the less-known wild American species may be proved to possess chromosomes homologous with those of the Asiatic species.

SUMMARY

The cytogenetic literature that has critical bearing on the relationships of species in the genus *Gossypium* is reviewed and summarized in table form.

The meiotic chromosome conjugation is reported of (1) F_1 of 23 new interspecific hybrids, including 12 different types of hybrids; (2) F_2 of certain 13- × 13-chromosome combinations; and (3) F_2 and F_3 of certain 13- × 26-chromosome combinations.

The conjugation observed, in conjunction with that previously reported, strongly supports the grouping of the species as follows: (1) The Australian species, *Gossypium sturtii;* (2) Asiatic species; (3) wild American species; (4) cultivated American species.

The limited meiotic chromosome conjugation of interspecific hybrids involving *Gossypium sturtii* indicates that there is very little, if any, homology within the haploid complement of *G. sturtii* and that its chromosomes are very slightly, if at all, homologous with chromosomes of any other species, with the possible exception of *G. davidsonii.*

The degree of meiotic chromosome conjugation in hybrids involving Asiatic species indicates: (1) That if homology exists within the haploid complement it is very limited; (2) that the cultivated Asiatic species are very closely related to one another; (3) that the wild African species *Gossypium anomalum* is rather closely related to the cultivated Asiatic species; (4) that the wild Asiatic species *G. stocksii* is not closely related to the cultivated Asiatic species and to *G. anomalum;* (5) that within the Asiatic group the relationship to the cultivated American species is closest in the cultivated Asiatic species and

most distant in *G. stocksii*, *G. anomalum* occupying an intermediate position; and (6) that the Asiatic species are not closely related to the wild American species.

Hybridization experiments, meiotic chromosome behavior in F_1, and histological observations indicate that the wild American group is composed of two, or possibly three subgroups: (1) *Gossypium armourianum* and *G. harknessii;* (2) *G. davidsonii* and *G. klotzschianum;* and (3) *G. thurberi*, which appears to be intermediate between subgroups 1 and 2 but more closely related to subgroup 1. *Erioxylum aridum* probably should be included in the genus *Gossypium* and in subgroup 3 of the wild American group. The degree of meiotic chromosome conjugation in hybrids involving wild American species denotes (1) that there is very little, if any, homology between the chromosomes within their haploid complement, and (2) that subgroups 1 and 3 are somewhat closely related to the cultivated American species. It also appears that the chromosomes of these species and of the Asiatic species are homologous with different sets of 13 chromosomes in the cultivated American species. There are no available data that indicate the relationship of *G. davidsonii* and of *G. klotzschianum* to either *G. stocksii* or the cultivated American species. The segregates from wild American species × cultivated American species apparently revert to 26-chromosome forms, which may, at times, resemble 26-chromosome species other than the corresponding parent of the hybrid.

The meiotic chromosome conjugation in haploids, in diploids, and in hybrids involving the cultivated American species indicates (1) that the chromosomes within the haploid complement of the cultivated American species are partly homologous and (2) that the various cultivated American species are closely related.

Three hypotheses as to the origin of 13-chromosome species are discussed, and evidence for and against each hypothesis is given.

Evidence is given that the 26-chromosome species are polyploids, and the possibility is discussed of there being, besides true allopolyploids, autopolyploids that behave as allopolyploids Evidence is stated for and against the hypothesis of the purely American origin of American cultivated cottons and the alternative hypothesis that they may have originated from the hybridization of an American with an Asiatic species.

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STUDIES OF THE NATURE OF ROOT KNOT RESISTANCE[1]

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INTRODUCTION

During the course of breeding experiments with beans (*Phaseolus vulgaris* L.) resistant to root knot caused by the nematode *Heterodera marioni* (Cornu) Goodey, two observations were made by the writer[3] which raised the question of the nature of root knot resistance. (1) Alabama No. 1 and Alabama No. 2 beans (*P. vulgaris*), which are highly resistant to root knot both in the seedling and adult stage, were observed to form occasional slight swellings immediately behind the root tip when grown in soil badly infested with nematodes. These swellings were never observed to develop into galls. This observation raised the question as to whether Alabama No. 1 and Alabama No. 2 beans resist the entry of nematodes into their roots with the exception of occasional larvae which induce these slight swellings, or whether larvae enter but fail to induce the formation of giant cells and the resulting galls. (2) In the adult stage the Hopi lima beans (*P. lunatus* L.) were consistently less susceptible to root knot under field conditions than other varieties of the species. In the seedling stage, however, no differences could be found between the degree of root knot infection on the Hopi strains and the most susceptible varieties. This suggested an adult plant resistance either of a physiological nature, possibly due to a substance toxic to the nematodes, or of a morphological nature, possibly associated with the tougher root tissue possessed by the Hopi limas.

In the experiments reported in this paper an attempt has been made to elucidate this problem, which is of fundamental interest both to nematologists and breeders working on problems of root knot resistance. The scope of the investigation has been expanded to include the nature of root knot resistance in general.

DEFINITION OF TERMS AND LITERATURE REVIEW

Steiner[4] distinguishes between host resistance to nematode infestation and host immunity. He considers resistant plants to be those that actually resist the entry of nematode larvae by some mechanical or chemical means. Host immunity on the other hand is considered to be the ability of certain plants to show few signs of suffering in spite of the presence of root knot galls. For the purpose of this investigation the term "root knot resistant" will be substituted for "nematode resistant," which has been used by most workers. Root

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[2] The writer is indebted to Dr G. Steiner, of the Bureau of Plant Industry, U. S. Department of Agriculture, for the loan of an unpublished manuscript by Dr. E. G. Arzberger on the comparative morphology of roots of resistant and susceptible cowpeas.

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knot resistant will be taken to mean any perceptible ability on the part of a plant to grow in nematode-infested soil without the formation of root knot galls. This entirely disregards the important but distinct problem of host tolerance, or host immunity, as it is called by Steiner. In this sense root knot immunity would be taken to mean complete resistance to root knot.

In only one instance, insofar as the writer can determine, has an attempt been made to account for resistance to the root knot disease. In an unpublished investigation Arzberger [5] compared the morphology of the roots of root knot resistant and susceptible cowpeas (*Vigna sinensis* (Torner) Savi). He found that two resistant varieties, Iron and Brabham, had roots that were better guarded by protective tissue than Whippoorwill and Early Buff, which are susceptible. In the resistant varieties the cork layer was also found to be better developed, with fewer broken areas, and the walls of the cork cells were more suberized. The mechanical tissue in the cortex of roots of resistant varieties was found to be more uniformly distributed. Susceptible varieties were found to contain more starch in the cortex, and cells containing starch were more remote from the periphery of the root.

EXPERIMENTAL METHODS AND RESULTS

A microscopical study of bean rootlets was conducted in order to determine whether nematode larvae actually enter root knot resistant varieties and, if so, at what rate as compared with susceptible varieties. Seeds of Alabama No. 1 and Kentucky Wonder beans were planted in 6-inch pots in the greenhouse. The soil was a sandy loam with which had been mixed approximately 20 percent by volume of nematode inoculum prepared by chopping up large root knot galls from adult common bean plants. The seeds were not planted until 3 days after the inoculum was added in order to give the larvae an opportunity to emerge from the root knot tissue and become distributed in the soil. When the secondary roots of the sprouting seeds were between 2 and 4 cm long the seedlings were removed intact by submerging the pots in water and floating them free. Ten vigorous plants each of Alabama No. 1 and Kentucky Wonder were washed free from clinging soil, and 10 rootlets were cut from each plant approximately 1 cm from the tip. The rootlets from each plant were then placed in separate glass vials and treated with hot acetone, followed by Flemming's strong fluid, according to the schedule suggested by Christie.[6] After dehydration, the material was cleared in clove oil and allowed to remain in this medium during the time the studies were being conducted. The larvae were stained black by this procedure, and it was found possible to observe them by the use of dissecting binoculars of the magnification of approximately × 40. In making routing observations the rootlets were floated in clove oil in a small Petri dish and by manipulation with dissecting needles were passed one at a time through the field of vision. This method permitted a rapid study of the material. Samples of these stained rootlets are shown in plate 1, *A* and *B*.

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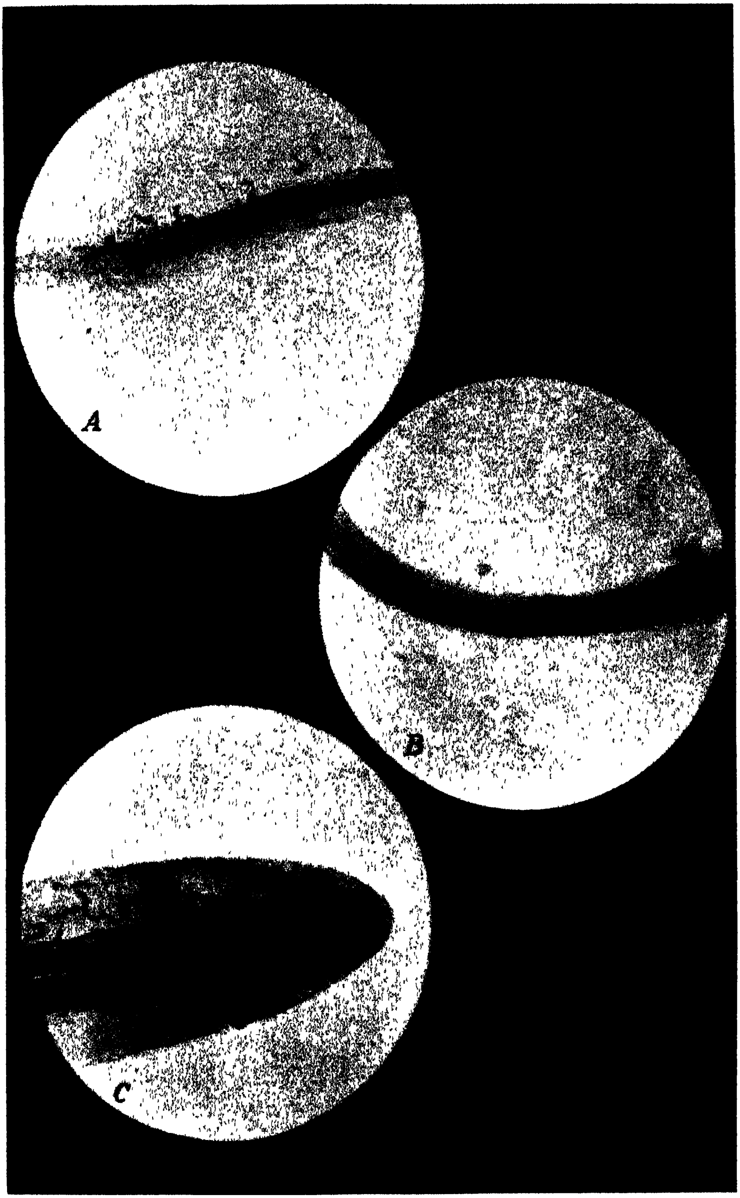

Root knot larvae in a root of (*A*) Trucker Favorite corn and (*B*) Henderson Bush lima bean. These preparations were stained with Flemming's strong fluid and mounted in balsam. Root tip of Alabama No. 1 bean (*C*) containing so many larvae that it was impossible to count them. The number in a 1-cm section of root was conservatively estimated at 90. Approximately ×44.

The number of larvae observed in each root of the 10 Alabama No. 1 and 10 Kentucky Wonder plants is given in table 1, together with the means for each plant and also the means for the two varieties. It will be seen from the table that considerable variation occurred in the number of larvae found in the rootlets from the same plant. The explanation of the relatively large number found in some rootlets is probably that these rootlets grew near or into a piece of inoculum harboring a large number of larvae. Other rootlets escaped infection altogether. It was not uncommon to observe a number of larvae at one point but nowhere else in the same rootlet.

The generalized standard error calculated on the basis of a 20-plant experiment with 10 replications is 37.66 percent. It will be noted that the mean number of larvae observed in the rootlets of Alabama No. 1 is only slightly lower than that observed in rootlets of Kentucky Wonder. Using the Fisher[7] *t* test for significance of differences, it is readily seen that this difference is not significant at the 5-percent level. Furthermore, using the same test, it is found that the means of no two plants studied differ significantly at the 5-percent level.

TABLE 1.—*Mean number of nematode larvae observed in 10 rootlets each of 10 plants of Alabama No 1 and Kentucky Wonder beans*

Variety and plant No	Larvae in root No —										Mean number per rootlet
	1	2	3	4	5	6	7	8	9	10	
Alabama No 1											
1	0	2	2	7	15	8	0	1	3	1	3.9
2	2	6	0	0	3	0	1	2	9	4	2.7
3	3	7	0	0	8	1	7	2	1	6	3.5
4	4	4	1	4	1	1	7	2	4	0	2.8
5	31	7	2	7	1	0	4	7	1	4	6.4
6	7	3	7	4	4	4	1	1	0	1	3.2
7	8	2	8	3	0	6	0	9	1	0	3.7
8	9	2	9	1	1	4	4	24	0	2	5.6
9	0	1	0	4	0	0	3	0	7	16	3.1
10	10	0	1	7	3	0	0	7	2	5	3.5
Mean											3.84
Kentucky Wonder											
1	0	8	3	9	2	7	7	0	1	34	7.1
2	1	4	6	8	7	8	1	3	0	7	4.5
3	3	9	2	1	5	10	3	4	3	0	4.0
4	8	6	4	16	0	9	4	1	6	4	5.8
5	0	1	4	0	1	7	2	0	7	9	3.1
6	9	3	0	0	0	6	0	1	2	8	2.9
7	3	4	7	8	0	2	1	5	1	0	3.1
8	0	1	3	4	0	1	3	6	0	1	1.9
9	1	0	4	3	7	7	4	0	3	2	3.1
10	1	3	7	1	0	8	7	3	6	4	4.0
Mean											3.95

Inasmuch as these data indicated that the root knot resistance of Alabama No. 1 bean is not due to the lack of entry or differences in the rate of entry of nematode larvae it appeared desirable to extend the experiment to include a number of varieties and species of plants of known resistance and susceptibility. Thirty varieties and species were studied, including the two beans used in the previous experiment. Counts were made of the number of nematode larvae in rootlets, using the same technique as previously described with the exception that the nematode inoculum used was prepared from badly diseased roots of common beans, lima beans, tomatoes, and cowpeas rather than common beans alone. The mean number of larvae observed in the rootlets of each species is presented in table 2. Considerable variation within species occurred as indicated by the relatively high standard-error values.

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TABLE 2.—*Mean number of nematode larvae observed in rootlets of 30 varieties and species of plants*

Response [1] to root knot and scientific name	Common name	Variety name	Total root lots studied	Mean larvae per rootlet [2]
			Number	*Number*
Resistant:				
Phaseolus vulgaris L.	Common bean	Alabama No. 1	28	4.6±1.3
P. lunatus L.	Lima bean	Hopi 155	30	5.0±1.0
Vigna sinensis (Torner) Savi.	Cowpea	Conch	21	5.8±1.2
Do	do	Iron	33	4.5±.8
Do	do	Brabham	32	7.2±1.6
Do	do	Victor	30	3.5±.9
Crotalaria spectabilis Roth.	Crotalaria		38	3.9±.7
Raphanus sativus L.	Radish	Scarlet Globe	23	5.1±1.1
Brassica japonica Siebold	Mustard	Giant Southern Curled	20	3.9±.9
B. rapa L.	Turnip	Purple Top White Globe	37	3.7±.5
Allium cepa L.	Onion	Prizetaker	26	4.5±1.3
A. vineale L.	Wild onion		20	4.1±1.0
Cosmos bipinnatus Cav.	Cosmos	Early White	21	3.5±1.1
C. sulphureus Cav.	Golden cosmos	Orange Flare	26	5.8±1.5
Zinnia elegans Jacq.	Zinnia	Lilliput Salmon Rose	29	6.3±1.1
Secale cereale L.	Rye	Abruzzi	30	4.8±1.2
Avena sativa L.	Oat	Hasting Hundred Bushel	33	3.4±.7
Triticum aestivum L.	Wheat	Alabama Bluestem	27	4.0±1.3
Zea mays L.	Corn	Trucker Favorite	20	5.4±1.2
Do	do	Golden Bantam	21	4.9±1.5
Sorghum vulgare Pers.	Sorghum	Sagrain	22	5.6±1.1
Sorghum halepense (L.) Pers.	Johnson grass		23	4.7±1.1
Cynodon dactylon L.	Bermuda grass		20	4.1±.8
Digitaria sanguinalis (L.) Scop.	Crabgrass		26	4.6±1.2
Mean				4.7±.2
Susceptible:				
P. vulgaris L.	Common bean	Kentucky Wonder	39	4.5±1.2
Do	do	Burpee Stringless Greenpod	23	7.8±2.4
P. lunatus L.	Lima bean	Henderson Bush	29	4.8±1.2
Vigna sinensis (Torner) Savi.	Cowpea	Cream Crowder	23	5.8±1.4
Lycopersicum esculentum Mill	Tomato	Marglobe	26	6.2±1.7
Hibiscus esculentus L.	Okra	Hasting Dwarf	22	4.1±1.2
Mean				5.4±.6

[1] The classification into resistant and susceptible varieties is based on observations of the writer and on various reports in the literature. Those species which show any appreciable signs of resistance are included in the resistant class.

[2] The standard-error values were calculated on an individual-variety basis. Standard-error values for the means of the resistant and susceptible varieties are for the mean of a series of means.

It will be seen from the data presented in table 2 that, in the species studied, no general relationship exists between the rate of larval entry and root knot resistance. Although the mean number of larvae observed in rootlets of susceptible species is slightly greater than that observed in rootlets of resistant species, there is no significant difference between these means at the 5-percent level.

There are no significant differences at the 1-percent level between the mean number of larvae observed in the rootlets of any two varieties or species. At the 5-percent level the mean for Brabham cowpea appears significantly higher than that for Victor cowpea and Hasting Hundred Bushel oats; however, no importance can be attached to this in view of the fact that all three are somewhat resistant to root knot. Furthermore, the high mean of Brabham cowpea is due to a few rootlets containing an exceptionally large number of larvae.

The question was raised whether the resistant varieties and species would develop galls if allowed to grow beyond the seedling stage in soil as severely infested as that used in these experiments. In order to investigate this point 10 seedlings each of a number of varieties studied in the preceding experiment were transplanted to heavily inoculated soil in a greenhouse bed. All seedlings used for this purpose were grown in the same soil and at the same time as those from which rootlets were removed for the preceding study. The resistant varieties tested in this way were Alabama No. 1 bean, Hopi 155 lima bean, Conch cowpea, rye, oats, and Trucker Favorite corn. Three susceptible varieties, Kentucky Wonder bean, Cream Crowder cowpea, and tomato, were included for purposes of comparison. Roots were examined 7 weeks after transplanting, and in every instance the extent of root-knot development agreed with numerous field observations. Many galls were found on the three susceptible varieties, and some galls of a small size were observed on Hopi 155 lima bean. A few very small galls were found on roots of Conch cowpea and Trucker Favorite corn, while rye and oats showed no evidence of nematode infestation with the exception of a few very slight swellings. Inasmuch as rootlets from other seedlings of these varieties grown at the same time and under exactly the same conditions showed no significant differences in the rate of larval entry, these differences in the development of root knot must be due to hereditary differences in ability to resist gall formation.

In order to determine whether resistance to the entry of nematode larvae might develop with age adult plants of a few resistant and susceptible varieties growing in pots were sunk in soil that had been thoroughly inoculated with nematodes. The roots were allowed to to grow through the bottom of the pot. Tips 1 cm long were then removed and stained as in the previous experiments. Twenty rootlets each of three susceptible and three resistant varieties were observed.

The mean number of larvae found in rootlets of each variety is recorded in the following tabulation:

Variety	Mean number of larvae per rootlet[1]
Resistant:	
Alabama No. 1 bean	7.1±4.5
Hopi 155 lima bean	3.0± .7
Trucker Favorite corn	3.1±1.2
Susceptible:	
Kentucky Wonder bean	4.3±1.2
Henderson Bush lima bean	2.7± .7
Marglobe tomato	4.0± .9

[1] The standard-error values were calculated on a single-variety basis.

It will be seen that as many larvae entered the rootlets of adult plants of Alabama No. 1 bean, Hopi 155 lima bean, and Trucker Favorite corn, three resistant varieties, as entered those of Kentucky Wonder bean, Henderson Bush lima bean, or Marglobe tomato, all of which are highly susceptible. It is interesting to note that one rootlet of Alabama No. 1 observed in connection with this study contained so many nematode larvae it was impossible to count them accurately. The estimated number was 90. A portion of this rootlet is shown in plate 1, *C*.

An experiment was conducted in order to determine whether Hopi 155 lima bean developed the ability to resist gall formation with age. Henderson Bush lima was used for purposes of comparison. Seeds of each variety were planted in 2½-inch pots in nematode-free soil on June 1, June 28, and July 12. The first two plantings were shifted to 4-inch pots when 2 weeks old. On July 26 the pots were lifted and sunk in inoculated soil, and on the same day seeds of both varieties were planted in the same inoculated soil in a greenhouse bench. Thus there were seeds and 2-, 4-, and 8-week-old plants all in the same lot of inoculated soil. Roots grew through the bottoms of all the pots within 4 days after setting. Therefore, roots of the different-aged plants including the seedlings were exposed to nematodes at approximately the same time. Roots of plants of different ages were examined from time to time, and all were removed 8 weeks after being exposed to nematode-infested soil.

The following observations were made during the course of this experiment on root knot development in Hopi 155 and Henderson Bush lima bean.

Three-week-old seedlings of the two varieties showed the same degree of root knot development, confirming earlier observations of the writer.[8]

Young roots of both varieties, including those which grew from seedlings and potted plants of various ages, developed root knot to the same degree.

As infected roots grew, the galls on Henderson Bush lima developed at a more rapid rate than those on Hopi 155.

At the end of 8 weeks roots of Henderson Bush lima had much larger galls than roots of Hopi 155. Galls on the former had coalesced to some extent, and many of them had begun to decay.

Galls on Hopi 155 were similar in size to those commonly found on the same variety in the field. For the most part they were nothing more than slight swellings and were never more than twice the diameter of the root on which they were borne. There were no signs of decay.

DISCUSSION AND CONCLUSIONS

Although the subject has not been discussed to any extent in the literature because of the lack of experimental investigations, it has probably been assumed by many workers that root knot resistance is due to a plant's ability to prevent the entrance of nematode larvae. Steiner[9] states: "In the study of plant-parasitic nemas, the resistance of a plant means that it opposes the entrance of the nemas by some mechanical or perhaps chemical means." Although Arzberger[10] did not state such a conclusion, it is quite evident that he assumed the morphological root characteristics he found correlated with resistance to nematode infestation actually prevented the entry of nematode larvae. Insofar as the writer can determine no concrete evidence that larvae do not enter the roots of resistant varieties has been presented.

The experiments summarized in tables 1 and 2 show that root knot resistance, in the representative varieties and species studied, is not due to the lack of entry of nematode larvae. No significant differences were noted between the rate of entry in the most resistant and in susceptible plants in the seedling stage. Even rootlets of onion, which has been said to be repellant to nematodes, contained as many larvae as other species. That no ability to resist entry is developed with age is indicated by the experiment summarized in the tabulation on page 267.

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[8] BARRONS, KEITH C. See footnote 3.

[9] STEINER, G. See p. 527 of citation mentioned in footnote 4.

[10] ARZBERGER, E. G. See footnote 5.

From the observations made on root knot development on roots of Henderson Bush and Hopi 155 lima beans at different ages it appears that the resistance of the latter is only partial in the adult plant, although it is very definitely resistant to a notable degree when compared with Henderson Bush. This confirms numerous field observations made by the writer. Although seedlings and young roots on adult plants of Hopi 155 appear fully as susceptible as similar roots of Henderson Bush, gall development seems to be arrested as the roots mature. The fact that galls on the two varieties are the same size in the seedling stage but later are much smaller on Hopi 155 indicates that something within the roots of this variety retards root knot development.

It is hoped that further investigations on the comparative morphology and physiology of resistant and nonresistant varieties and species will make possible a better understanding of this problem. It seems reasonable to conclude on the basis of the data presented in this paper that resistance is due to factors entirely within the plant itself that do not exclude the entrance of larvae but either (1) kill the nematode after it enters or (2) cause it to starve. Nematologists are generally agreed that nematodes feed on the contents of the giant cells the formation of which is apparently induced by salivary secretions of the nematode itself. In view of the adverse conditions which nematodes have been known to withstand in experiments it seems more likely that their lack of development in resistant plants is a matter of starvation rather than death by some lethal agent. Although it is not impossible that certain morphological root characteristics accomplish or contribute to resistance, it seemed more reasonable to postulate resistance due to certain physiological factors.

As a speculative hypothesis the writer suggests that resistance may be due to certain chemicals within the roots of resistant plants that counteract or neutralize the giant-cell-inducing effect of the salivary secretions of the nematode. It is believed possible to account for the known facts concerning resistance with such a hypothesis. For example the occurrence of varietal differences in cowpeas could be thus explained. Isbell [11] has found that Conch is highly resistant to root knot, showing only occasional galls, while Cream Crowder is very susceptible. Between these two extremes are varieties possessing various degrees of resistance. It seems possible that these varietal differences may be due to their varying ability to synthesize the hypothetical neutralizing substances. Conch has been observed to form an occasional root knot gall. This variety may synthesize these substances in quantities sufficient to neutralize the secretions of practically all larvae. However, if an enormous number of larvae entered the root at one point, it is possible that neutralizing substances would not be present in quantities sufficient to counteract their combined effect. Under such circumstances giant cells would be formed, but the resulting gall would never attain the size of those on susceptible varieties formed under the same circumstances because of the continual synthesis of the neutralizing substances. This same explanation may account for the occurrence of occasional galls on a number of species that are generally considered resistant.

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[11] ISBELL, C. L. NEMATODE INJURY TO THE ROOTS OF TABLE VARIETIES OF COWPEAS. Ala. Agr. Expt. Sta. Ann. Rept. 45: 29-30. 1934.

This hypothesis conforms with the observed facts of adult plant resistance. As previously mentioned, seedlings and young roots of adult plants of Hopi 155 lima bean grown in nematode-infested soil develop root knot to the same degree as Henderson Bush lima while galls on mature roots of the former variety are much smaller and do not decay as readily. It is possible that the neutralizing substances are lacking in the young roots of Hopi 155 but are synthesized as the roots mature, thus inhibiting the development of the nematodes and thereby reducing the extent of gall formation. According to this hypothesis Henderson Bush lima and other highly susceptible plants lack the ability to synthesize these substances. Some plants show few, if any, signs of gall formation in the seedling stage. As mentioned earlier in this paper, only occasional slight swellings are found on the rootlets of Alabama No. 1 bean. This observation can fit into the neutralizing-substance concept by postulating that such varieties possess the ability to synthesize these substances in relatively large quantities at an early age.

The results of these experiments raise the question whether the phenomenon of host specialization in the root knot nematode as discussed by Steiner [12] involves differences in ability to perceive and enter the root or in ability to induce giant-cell formation once the larvae have entered. It is believed that most workers have held the former concept, but it seems possible that variations in the response of different nematode populations to the same plants may be due to differences in the salivary secretions of the nematode larvae. Possibly the gradual adaptation of a population to a specific host is a matter of genetic changes in the chemical nature of its secretions rather than changes in ability to perceive and enter the host. It is hoped that this fundamental problem which is of interest both to breeders and nematologists will be solved by further investigations.

The results of the above experiments indicate that the use of highly susceptible plants as trap crops may be unnecessary. If as many larvae enter resistant plants in which the nematodes will not go through their life cycle, why are not such plants just as effective in reducing nematode populations as the highly susceptible crops that will permit the population to increase unless properly handled? Although theoretically sound, the trap-crop method of nematode control has not proved practical, partly because of the necessity of plowing under or removing the infested plants before the first life cycle had been completed. In the light of these investigations there appears to be no reason why resistant varieties may not be used as trap crops. The resistant crops that have been used in rotations for the purpose of reducing nematode populations have probably been acting as traps.

[12] STEINER, G. See footnote 4.

SUMMARY

A microscopical study was made of rootlets from seedlings of Alabama No. 1 and Kentucky Wonder beans. It was found that as many larvae entered the rootlets of the former variety, which is highly resistant, as of the latter, which is susceptible to root knot.

A further microscopical study of the rootlets of a number of susceptible and resistant varieties and species of plants indicated that there were no significant differences in the rate of larval entry either in the seedling or adult stage. It had previously been assumed by a number of workers that resistant plants actually resisted the entry of nematode larvae.

Hopi 155 lima has been observed to be somewhat resistant to root knot in the field, but it was found that roots of seedlings of this variety developed root knot to the same degree as seedlings of Henderson Bush lima when grown in artificially inoculated soil. Experimental observations on plants of different ages indicated that young roots of Hopi 155 were susceptible regardless of the age of the plant on which they were growing. However, as the roots matured, the galls on Henderson Bush developed at a more rapid rate. Galls on Hopi 155 never grew very large and were less subject to decay than those on the roots of susceptible lima beans.

The hypothesis is proposed that root knot resistance is due to substances synthesized by the plant that counteract the giant-cell-inducing effect of the salivary secretions of nematode larvae. Such a hypothesis accounts for the known facts concerning hereditary resistance.

The results of these experiments raise two questions of fundamental importance: (1) Whether the phenomenon of host specialization may not be due to genetic differences in the chemical nature of salivary secretions and the resulting ability to induce giant-cell formation rather than differences in ability to perceive and enter the host; and (2) whether resistant plants may not be superior to susceptible plants as trap crops, inasmuch as equal numbers of larvae appear to enter the roots of resistant plants but do not reproduce.

EFFECT OF TEMPERATURE OF DIGESTION, CHEMICAL COMPOSITION, AND SIZE OF PARTICLES ON PRODUCTION OF FUEL GAS FROM FARM WASTES[1]

By G. H. NELSON, *cooperative agent,* ROBERT P. STRAKA, *associate bacteriologist,* and MAX LEVINE, *cooperative agent, Industrial Farm Products Research Division, Bureau of Chemistry and Soils, United States Department of Agriculture*

INTRODUCTION

Research on the anaerobic decomposition of farm waste for the production of methane and other gases for use as fuel on the farm has been stimulated by the development of a method of treating sewage in which the settleable organic solids are digested anaerobically by bacteria. Large quantities of gas having a relatively high calorific value are produced by this method. There are many difficulties, however, in the application of this sewage-sludge digestion process to fibrous farm wastes. Their tendency to lie close to or on the surface of liquid media in which they are suspended introduces a particularly difficult mechanical problem, and their low nitrogen content, in contrast to sewage sludges, makes it necessary to provide additional nitrogen.

Boruff and Buswell (*4, 7 8*)[2] reported the production of about 5 cubic feet of gas per pound of cornstalks, about 4.8 cubic feet per pound of flax straw, 3.4 cubic feet per pound of flax shives, 10.8 cubic feet per pound of filter paper, 5.8 cubic feet per pound of banana skins and stems, 3.9 cubic feet per pound of wheat straw, and 4.8 cubic feet per pound of newspaper.

Hussey, Row, and Allison (*9*), in a study of the utilization of cellulosic wastes in conjunction with sewage for the purpose of augmenting the gas supply, report but 1.5 cubic feet of gas per pound of peanut hulls, 3.9 cubic feet per pound of leaf waste, and 10.7 cubic feet per pound of wastepaper added to the sewage sludge.

The purpose of the investigation reported in this paper was to ascertain the effect of (1) temperature of digestion, (2) chemical composition, and (3) size of particles on the rate and quantity of gas produced from fibrous farm wastes by anaerobic microbial decomposition.

MATERIALS EMPLOYED

Particular attention was paid to cornstalks, wheat straw, artichoke tops, and seed flax straw. The cornstalks were used in the form of flour and as chopped material. The wheat straw was used as ground (1 mm) and as chopped material. In addition to these typical farm wastes filter paper and alkali lignin obtained from corncobs were employed in a few experiments to ascertain the digestibility of prepared cellulose and prepared lignin, two constituents characteristic of the wastes in question. Another substance used, referred to as "cook liquor," was the liquid residue left after digesting cornstalks with

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[1] Received for publication September 12, 1938

[2] Italic numbers in parentheses refer to Literature Cited, p. 286.

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alkali for the purpose of manufacturing wallboard. This liquid residue was evaporated to the consistency of a heavy paste, when it took on the appearance of blackstrap molasses. Cook liquor was included in the investigation because it was thought that the information obtained might be of use in the problem of treating wastes from wallboard factories should this industry develop to a point where numerous small plants would be located in the farm areas.

PROCEDURE

In general the procedure for preparation of an active seed was to add a little cornstalk flour to a mass of partly digested sewage sludge and allow digestion to go practically to completion, after which additional quantities of cornstalk flour were added. After several such additions the digested material was used as the seed for the various experiments.

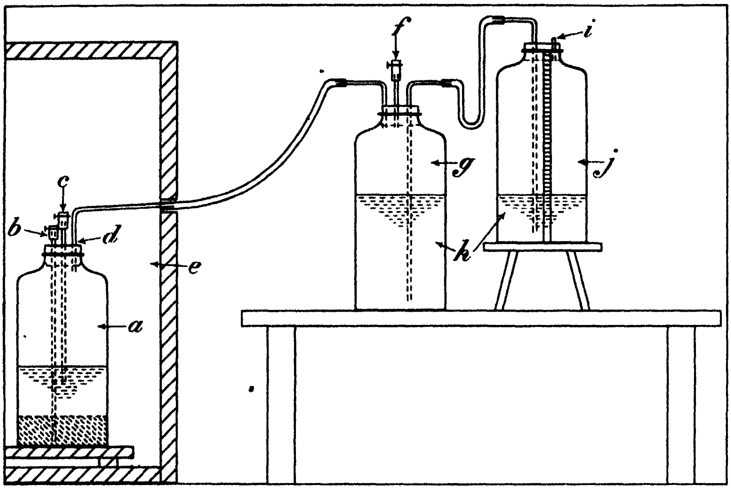

Figure 1.—Fermentation apparatus: *a*, Digestion bottle; *b*, tube for removing samples; *c*, tube for adding chemicals; *d*, tube to gas collector; *e*, incubator; *f*, tube for gas samples; *g*, gas-collecting bottle; *h*, saturated salt solution; *i*, air vent; *j*, bottle for measuring gas.

To a given volume of seed in a suitable container was added a sufficient quantity of test material to give the desired ratio of freshly added volatile solids[3] to volatile solids in the seed. This ratio was approximately 1 to 1 except in a few experiments at the high temperature (50°–55° C.), where the amount of volatile solids added was increased until the ratio was approximately 2.5 to 1. The ratios of fresh volatile solids to volatile solids in the seed for each of the materials studied are shown in table 2.

Each material was tested at digestion temperatures of 28° to 30° C. and 50° to 55°. The two series might be referred to as the mesophilic and thermophilic series, respectively.

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[3] The term "volatile solids" as used throughout this paper refers to the material lost when materials were heated in a furnace at 600° C.

The volume of the fermenting mixtures ranged from about 1,000 to 5,500 cc. As no significant differences in rate of gas production due to differences in volume were observed, the volumes are not recorded in the tables.

The digestion vessels consisted of wide-mouth glass bottles fitted with three-hole rubber stoppers arranged as shown in figure 1. Through one of these holes a ½-inch glass tube extended down to within a few inches of the bottom of the liquid. This was used for removing small samples from time to time to determine the change in reaction. A second tube, which extended through the stopper into the liquid, was used for the addition of ammonium salts and other reagents when necessary. The third tube which extended just through the rubber stopper, connected the digestion bottle with the bottle for the collection of gas.

In the experiments with filter paper and artichoke tops lime was added several times after the fermentation had started in order to prevent excessive acidity. The total nitrogen content in all these substances was low, running from zero in the filter paper to 0.68 percent in the cook liquor. If not already in the seed, an adequate amount of nitrogen was added in the form of ammonium phosphate. The total nitrogen (as ammonia and organic nitrogen) in the fermenting mixture ranged from 500 to 2,500 parts per million.

The analyses of the various materials employed are shown in table 1.

TABLE 1.—*Analyses of farm wastes used*

Material	Moisture [1]	Total solids [1]	Volatile solids	Ash [1]	Pentosans [2]	Cellulose [3]	Lignin [4]	Other material	Ash [1]	Total N_2 [1]
	Percent	*Percent*	*Percent*	*Percent*	*Percent* [5]	*Percent* [5]	*Percent* [5]	*Percent* [5]	*Percent* [5]	*Percent* [5]
Lignin	3.07	96.93	96.87	0.06	0.758	0	87.37	11.81	0.07	0.529
Filter paper	2.78	97.22	97.22	0	0	100.00	0	0	0	0
Cook liquor	56.91	43.09	37.02	6.07	28.58	0	24.10	33.22	14.10	.68
Cornstalk flour	5.22	94.78	86.90	7.88	29.72	33.55	18.49	9.93	8.31	.269
Artichoke-top flour	6.36	93.64	90.25	3.39	14.74	22.50	11.54	47.60	3.62	.435
Chopped cornstalks	3.46	96.54	90.15	6.39	26.92	34.90	18.55	13.02	6.61	.32
Chopped wheat straw [6]	4.71	95.29	88.20	7.09	28.60	33.60	15.37	14.96	7.47	.329
Ground wheat straw [6]	5.53	94.47	88.04	6.43	27.65	34.05	18.92	12.63	6.75	------
Seed flax straw	5.38	94.62	89.46	5.16	19.97	49.55	21.65	3.37	5.46	------

[1] Analysis by American Public Health Association and American Water Works Association (*2*).

[2] Association of Official Agricultural Chemists method (*3, p. 344*).

[3] Norman-Jenkins method (*12*).

[4] Method of Bray (*6*), as modified by Peterson, Walde, and Hixon (*13*).

[5] On dry basis.

[6] The samples of chopped and ground wheat straw were taken from different batches When the nature of the material, possible varieties, and different harvesting methods are taken into consideration, divergencies in composition of the magnitude in this table are not large.

It will be noticed from table 1 that the cook liquor and artichoke-top flour were particularly high in constituents other than pentosans, cellulose, and lignin. These constituents are for the most part water soluble and probably are carbohydrates. The percentages of pentosans and cellulose in the wheat straw and cornstalks were practically the same. The seed flax straw, on the other hand, differed strikingly from the other fibrous farm wastes in that it contained considerably less pentosans but more cellulose.

RATE AND QUANTITY OF GAS PRODUCED BY MATERIALS TESTED

The results obtained in the various experiments are given in table 2 and shown graphically in figure 2. The relation of temperature to digestion and the rate of gas production for the various substances tested are shown in figure 3. The results obtained with each substance will be discussed briefly.

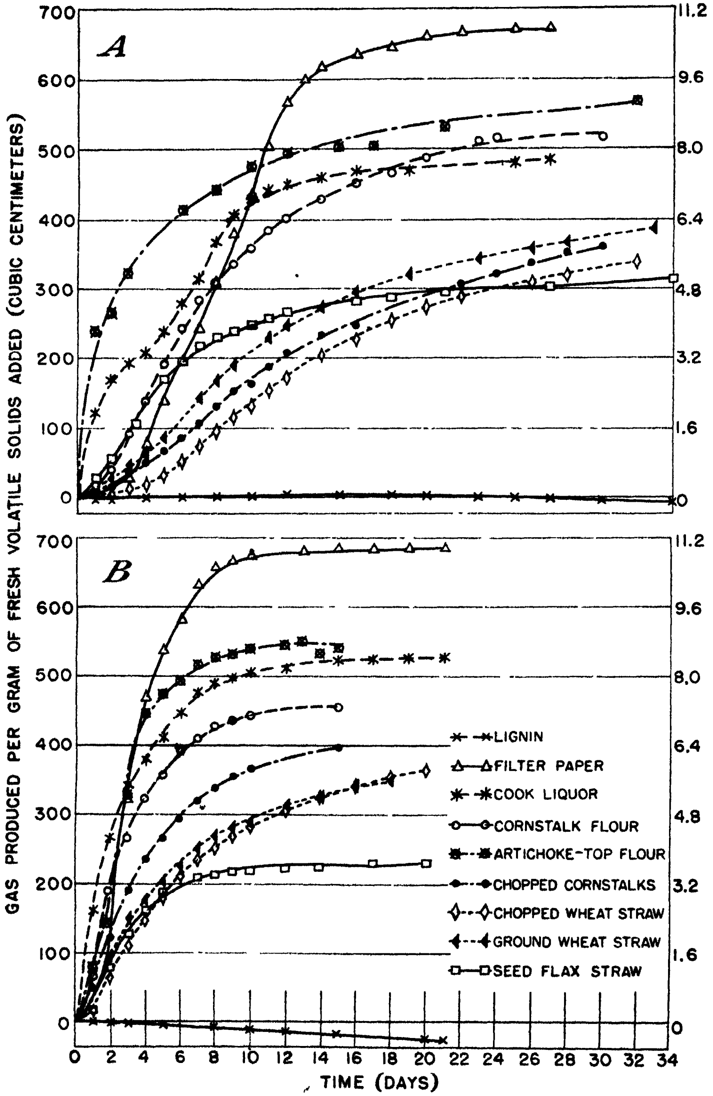

FIGURE 2.—Gas from farm wastes digested at: *A*, 28° to 30° C; *B*, 50° to 55°

LIGNIN

The lignin used was the so-called alkali lignin prepared from corncobs. The authors hoped to develop a flora that would be particularly effective in attacking lignin, but as may be seen from the results both at the low temperature (28° to 30° C.) and at the high temperature (50° to 55°), gas was not obtained when lignin was added to a ripe and active seed. As shown in table 2, the total amount of gas produced in the lignin-seed mixture was actually less than that produced from the seed control. It is evident that under the conditions of these experiments the digestion of lignin did not produce gas to any appreciable extent. The gas produced was low in methane (16.5 to 19.7 percent) and very high in hydrogen (16.4 to 16.6 percent). The hydrogen figures in this and the other series are probably higher than they would be in large-scale operation because of the temporary aerobic condition at the beginning of fermentation.

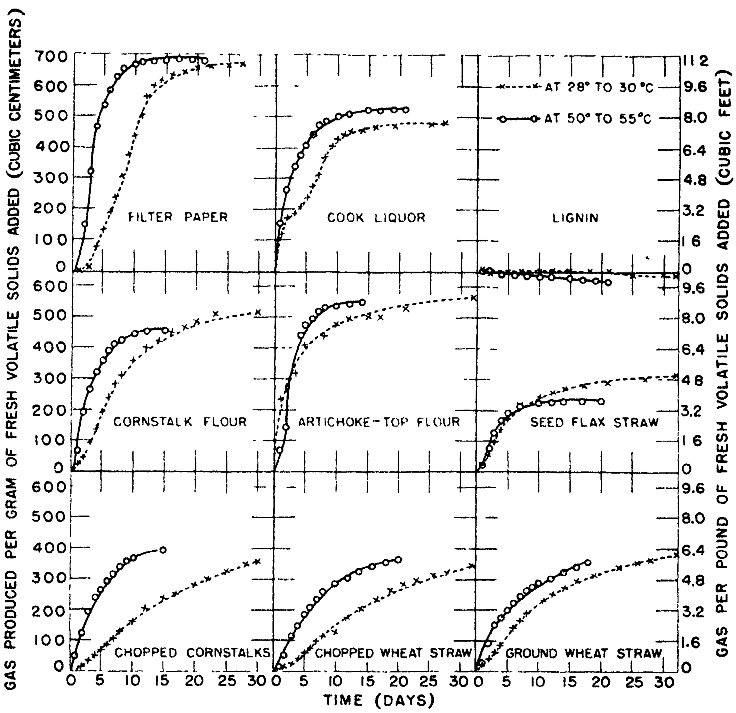

FIGURE 3.—Comparison of gas production at 28° to 30° and at 50° to 55° C.

FILTER PAPER

A high-grade Whatman quantitative filter paper was used in the experiments. Two runs were made at each of the temperatures. During one of the runs at the lower temperature repeated additions of small quantities of paper were made. The volume of gas produced in 27 days at 28° to 30° C. was 674 cc per gram of filter paper added.

TABLE 2.—*Gas from farm wastes digested at 28° to 30° and at 50° to 55° C.*

28° TO 30° C.

Material	Quantity used	Ratio of volatile solids added to volatile solids in seed	Runs	Gas produced per gram of volatile solids added																		
				1 day	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days	10 days	11 days	12 days	13 days	14 days	15 days	16 days	17 days	18 days	19 days
	Grams		*Number*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*
Lignin (alkali) [1]	36-70	0.84-1 06	4	−1.8	−2.4	0.7	1 3	1 4	1 6	2 2	2 6	2 3	4 5	4 9	4 6	4 4	------	4.0	3.6	------	4.2	3 5
Filter paper	36	.85	2	2	------	18 1	78 0	138 0	194 3	240 4	310 3	378.4	438 7	500.9	568 0	601 0	618 0	------	633.0	637.6	645.3	658.9
Cook liquor	38 15	.79	2	120 0	171 8	189 4	207 3	239 4	278.8	314 0	371 8	405.4	430 4	441 9	450 4	454.6	457.9	------	469.3	------	------	471.0
Cornstalk flour	25-100	.85-1 06	6	24 1	40.7	90 5	140.2	194 4	243 4	284 2	312 8	335 8	359 1	383.2	403 0	416 9	428 3	441 8	451 0	459.6	469 0	474.7
Artichoke-top flour	25	1 09	2	238 0	266 2	321 8	------	------	413 3	------	444 9	------	478 5	------	495 3	------	------	503 7	------	505.9	------	------
Chopped corn-stalks	25-75	1.05	2	4.8	15 6	37.3	50 4	67 7	87 4	108 0	130 1	151 4	163 6	186 8	206 6	221 6	230.6	239 6	246 1	252.7	262.5	272.3
Chopped wheat straw	69-100	1 01-1.04	3	------	11 1	15 3	24 9	36 4	57 8	78 6	98 2	116 9	135 3	157 8	175 8	------	208 4	------	233 4	243.9	------	26 3 2
Ground wheat straw	71-100	1 01-1 04	4	------	29 3	46 9	65 4	88 4	------	141 9	165 8	191 2	------	232 8	250 4	------	276 1	------	294.3	------	------	31 6.3
Seed flax straw	100	1 01	2	22 5	54.4	94 4	137 8	171 9	199 4	220 8	232 3	242 0	249 8	258 4	264 2	------	274 0	------	281 1	287.1	------	------

Material	Quantity used	Ratio of volatile solids added to volatile solids in seed	Runs	Gas produced per gram of volatile solids added															Gas produced per gram of total solids added	Composition of gas		
				20 days	21 days	22 days	23 days	24 days	25 days	26 days	27 days	28 days	29 days	30 days	31 days	32 days	33 days	34 days		CO_2	H_2	CH_4
	Grams		*Number*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Percent*	*Percent*	*Percent*
Lignin (alkali) [1]	36-70	0 84-1.06	4	2 9	3 8	------	2.6	------	−0 4	------	−1 1	------	------	−2 4	------	−3.9	------	−5 8	−5 79	13 3	16.6	19.7
Filter paper	36	85	2	662 7	------	664 5	------	------	669 0	------	673 6	------	------	------	------	------	------	------	673 6	38 5	4.2	49 4
Cook liquor	38 15	79	2	479 3	------	------	------	------	479 0	------	483 6	------	------	------	------	------	------	------	416 0	32 2	3 7	59.3
Cornstalk flour	25-100	.85-1 06	6	487 3	493 4	------	512 1	516 0	------	------	------	------	------	517 9	------	------	------	------	475.0	38 5	4 4	53 7
Artichoke-top flour	25	1 09	2	------	533 1	------	------	------	------	------	------	------	------	------	------	572.5	------	------	551 5	38 2	8.6	49.1
Chopped corn-stalks	25-75	1 05	2	282 0	293 1	304 3	312 3	320 4	328 3	336 1	343 9	351 9	355 8	359 7	------	------	------	------	336 0	32 4	8 8	53 9
Chopped wheat straw	69-100	1 01-1 04	3	------	280 1	------	295 6	------	------	312 2	------	322 6	------	------	------	342 7	------	------	317 2	36 0	6.2	55 4
Ground wheat straw	71-100	1.01-1 04	4	------	------	------	341 8	------	------	356 3	------	367 8	------	------	------	------	388 5	------	362.5	36.5	4.7	55 3
Seed flax straw	100	1 01	2	------	292 9	295 1	------	------	------	------	305 3	------	------	------	------	------	------	316 9	300.0	37.4	1.1	58 8

50° TO 55° C.

Material	Quantity used	Ratio of volatile solids added to volatile solids in seed	Runs	Gas produced per gram of volatile solids added												
				1 day	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days	10 days	11 days	12 days	13 days
	Grams		*Number*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*
Lignin [1] (alkali)	32. 1–116. 0	0. 88–1 10	4	1. 09	8. 57	2 3	−1. 13	−2 58	−5. 10	−7. 8	−10. 6	−11 9	−13 3	−15 2	−15. 3	−16. 4
Filter paper	20	1. 06	2	7. 9	150 2	320 1	469 8	537. 4	587 8	631 7	655 5	666 2	674 5	678 7	--------	679 8
Cook liquor	20	. 95	2	160 0	264 2	340 4	379. 8	411 6	444 7	475 6	487. 4	498. 0	507. 6	--------	511. 2	515. 2
Cornstalk flour	12. 4–127 0	. 97–1 30	8	69 1	193 2	269 1	324 4	359. 7	397 9	416. 8	428 2	436. 8	446 9	--------	454. 0	454. 6
Artichoke-top flour	23. 3	50	2	71 9	142 9	324 2	448 6	477. 2	493 5	520 6	530 3	531 8	539 7	544 1	545. 1	528. 9
Chopped cornstalks	25 0–48 7	1. 02–1 35	4	51 0	124. 2	194 0	239 8	268. 6	296 7	322 8	342 1	360. 5	369. 7	--------	--------	--------
Chopped wheat straw	14. 5–165. 0	1. 00–2. 58	3	18 6	69. 4	116 2	148 3	184. 8	211 5	238 4	261. 7	273 6	285. 8	--------	309 0	322 2
Ground wheat straw	15 0–30. 0	1. 03–2 58	3	31 3	96 8	154 6	175 3	202. 8	222 6	247. 4	266 2	277 8	288. 4	297 1	305 8	313 7
Seed flax straw	25	2 05	3	21. 8	78 2	129 7	166 6	191 2	--------	211. 5	216. 2	220. 2	222. 9	222 9	223. 6	225. 7

Material	Quantity used	Ratio of volatile solids added to volatile solids in seed	Runs	Gas produced per gram of volatile solids added								Gas produced per gram of total solids added	Composition of gas		
				14 days	15 days	16 days	17 days	18 days	19 days	20 days	21 days		CO₂	H₂	CH₄
	Grams		*Number*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Percent*	*Percent*	*Percent*
Lignin [1] (alkali)	32 1–116. 0	0 88–1. 10	4	−17. 8	−18 9	−20 2	−21 6	−22 1	−23 0	−23 9	−29. 9	−29 8	21 5	16. 4	16. 5
Filter paper	20	1 06	2	--------	684 6	--------	687 0	--------	687. 4	--------	687 7	687. 7	40 3	. 7	55. 7
Cook liquor	20	. 95	2	--------	521 9	--------	525. 2	--------	527. 2	--------	528. 5	454 0	34 5	1. 9	56. 8
Cornstalk flour	12 4–127 0	. 97–1. 30	8	--------	455 9	--------	--------	--------	--------	--------	--------	419 0	32 4	10. 3	53. 5
Artichoke-top flour	23 3	50	2	538 6	--------	--------	--------	--------	--------	--------	--------	519 0	28. 1	11 0	52. 6
Chopped cornstalks	25 0–48 7	1 02–1 35	4	--------	395 7	--------	--------	--------	--------	--------	--------	369 5	35 5	3 6	55. 9
Chopped wheat straw	14 5–165 0	1 00–2 58	3	328 1	334 8	341 1	348 5	354. 1	360 8	364 8	--------	338 0	29 9	8. 8	54. 1
Ground wheat straw	15 0-30. 0	1 03–2 58	3	327 0	335 9	343 4	--------	354 5	--------	--------	--------	331. 0	28. 1	7 0	57 3
Seed flax straw	25	2 05	3	227 0	--------	--------	230. 3	231 0	231. 0	231 0	--------	218. 5	27. 1	2. 7	55 7

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[1] Minus sign indicates that less gas was produced from the lignin and the seed than from the seed alone

This is equivalent to 10.8 cubic feet of gas per pound of filter paper added. Ninety percent of the gas was produced, however, in 14 days (*1, 2, 3, 4, 6, 8*).

At the high temperatures the rate of gas production was considerably greater; a total of 688 cc per gram of filter paper added, or 11 cubic feet per pound, was produced in 21 days. At this temperature 90 percent of the gas was produced in 6.8 days, or about half the time in which a similar quantity of gas was produced at 28° to 30° C.

For the purpose of comparing the effect of temperature on the rate of gas production, the time required to produce a definite volume, 300 cc per gram of volatile solids added, at each temperature range was determined for each material. The time required for filter paper to produce this volume of gas was 7.8 days at 28° to 30° C. and about 2.9 days at 50° to 55°. The higher temperature reduced the digestion time 63 percent.

The mixture had a marked tendency to become acid. This was particularly true at the low temperature.

The gas contained approximately 49.4 percent of methane at the low temperature and 55.7 percent at the higher temperature. The hydrogen content was 4.2 and 0.7 percent, respectively. Here, again, it is desired to point out that in large-scale operation the methane content would probably rise to 55 to 60 percent.

COOK LIQUOR

Two runs of the cook liquor were made at each temperature. During one of the low-temperature runs repeated additions of small quantities of material were made. During a digestion period of 27 days at 28° to 30° C., 484 cc of gas per gram of volatile solids added was produced; 90 percent of the gas was produced in approximately 11 days. At a temperature of 50° to 55° the volume of gas produced in 21 days was 529 cc per gram of volatile solids added, 90 percent of this gas being produced in 7 days. The volume of gas produced per pound of volatile solids added as cook liquor was 7.6 cubic feet at 28° to 30° and 8.45 cubic feet at 50° to 55°.

The time required to produce 300 cc of gas per gram of volatile solids added was 6.5 days at the lower temperature and 2.5 days or 62 percent less, at the higher temperature.

The gas produced contained 59.3 percent of methane at the lower temperature and 56.8 percent at the higher temperature. There was no significant difference in the nature of the gas evolved at the two temperatures.

ARTICHOKE TOPS

Artichoke tops were ground to a flour, and two runs were made at each of the temperatures. As in the previous series, one of the runs at the lower temperature consisted of repeated additions of small quantities of artichoke-top flour.

At 28° to 30° C., 573 cc of gas per gram of volatile solids added was produced in 32 days, and at 50° to 55°, 539 cc was produced in 14 days. This corresponds to 9.2 and 8.6 cubic feet per pound of volatile solids added at the low and high temperatures, respectively. Ninety percent of the gas was produced at the lower temperature in 19 days and at the higher temperature in about 6 days. The period for production of 300 cc of gas per gram of volatile solids added was

2.4 days at 28° to 30°, as compared with 2.5 days at 50° to 55°. This is in marked contrast with the results in the filter-paper and cook-liquor experiments, where the higher temperature resulted in much more rapid production of gas.

The gas evolved contained 49.1 percent of methane at 28° to 30° C. and 52.6 percent at 50° to 55°.

CORNSTALKS

The cornstalk series included six runs with cornstalk flour and two runs with chopped cornstalks at 28° to 30° C., and eight runs with cornstalk flour and four runs with chopped cornstalks at 50° to 55°.

The cornstalk flour, which had a fineness of approximately 200 mesh, was made by grinding cornstalks in a rod mill. The chopped cornstalks, which passed a ½-inch screen but were retained by a ¼-inch screen, were cut in a silage shredder.

The volume of fermenting materials in the various runs ranged from 1,500 to 5,500 cc, but there were no significant differences in the rate of gas production. In the cornstalk-flour series at the lower temperature 518 cc of gas per gram of volatile solids added was produced in 30 days, as compared with 456 cc in 15 days at 50° to 55° C. This corresponds to 8.3 cubic feet per pound of volatile solids at 28° to 30° and 7.3 cubic feet at 50° to 55°. Ninety percent of the gas was produced in a little more than 17 days at the lower temperature and in somewhat less than 7 days at the higher temperature. The time required to evolve 300 cc of gas was 7.5 days at 28° to 30° and 3.5 days at 50° to 55°, or approximately 53 percent less at the higher temperature.

The gas evolved at 28° to 30° C. contained 53.7 percent of methane, as compared with 53.5 percent at the higher temperature.

The rate of gas production by the chopped cornstalks was very much slower. At 28° to 30° C., 360 cc of gas per gram (5.76 cubic feet per pound) of volatile solids added was produced in 30 days, while at 50° to 55°, 396 cc per gram (6.3 cubic feet per pound) was produced in 15 days. At the lower temperature 90 percent of the gas evolved was produced in 24.5 days; at the higher temperature the same percentage of gas was produced in about 8.5 days.

The time required for the production of 300 cc of gas per gram of volatile solids added was 22 days at the lower temperature and 6 days at the higher temperature, or approximately 73 percent less at the higher temperature. The gas contained 53.9 percent of methane in the experiments at 28° to 30° C. and 55.9 percent in the 50° to 55° series.

Figure 3 shows the influence of size of the cornstalk material on the rate of digeston at the two temperatures. It will be noted that the chopped cornstalks and the cornstalk flour were decomposed rapidly at the higher temperature, the time required to produce 300 cc of gas being 6 days for the chopped material and 3.5 days for the flour. The time required for the chopped cornstalks was not quite twice that required for the flour. At the lower temperature the chopped cornstalks required 22 days and the flour 7.5 days to produce this quantity of gas. At this temperature nearly three times as long a digestion period was required for the chopped cornstalks as for the cornstalk flour. It is suggested that the difference in the relative rates of gas production by the chopped cornstalk and the flour at the two temperatures was probably due to an increased rate of penetration of the enzymes into the chopped material at the higher temperature.

The results also indicate that the effect of temperature depends not only on the chemical composition of the material undergoing digestion, for the two materials were practically identical chemically, but also on its physical character, as has been mentioned by Norman (*11*).

WHEAT STRAW

The wheat straw series consisted of straw that had been chopped or ground by means of a hammer mill. The chopped straw consisted of particles about one-quarter of an inch long and the ground straw of particles about one twenty-fifth of an inch in diameter.

It will be noted from table 2 that in some of the experiments at 50° to 55° C. the quantity of volatile solids added as wheat straw was more than two and a half times that in the seed, whereas at the lower temperature the ratio was always 1 to 1. It was thought that this larger proportion of fresh volatile solids might have slowed up the rate of gas production at the higher temperature, but examination of the individual runs disclosed that the rate of gas production was not slower in those experiments in which the larger proportion of volatile solids was employed.

In 28 days' digestion at 28° to 30° C. the gas produced per gram of volatile solids added was 323 cc for the chopped wheat straw and 368 cc for the ground wheat straw. This corresponds to a gas volume of 5.2 and 5.9 cubic feet, respectively, per pound. At the higher temperature the volumes of gas produced by the chopped and ground materials in 18 days were almost identical, 354 and 355 cc, which corresponds to 5.7 cubic feet per pound of volatile solids added. Ninety percent of the gas evolved was obtained at the lower temperature in 24 to 25 days and at the higher temperature in approximately 13 to 14 days.

The ground wheat straw produced 300 cc of gas per gram of volatile solids added in approximately 16.5 days at the lower temperature and in 11.5 days at the higher temperature. The digestion period was decreased about 30 percent by employing the higher temperature.

For chopped wheat straw the time required for the production of 300 cc of gas was approximately 25 days at 28° to 30° C. and 11 days at 50° to 55°. In this case the higher temperature decreased the digestion period about 56 percent. The effect of temperature on the rate of digestion was more marked with the chopped than with the ground material. This was also noted in the cornstalk series.

The amount of methane in the gas was approximately the same in all the series, 55.3 and 55.4 percent being present in the ground and chopped straw series, respectively, at 28° to 30° C. and 57.3 percent and 54.1 percent at 50° to 55°.

SEED FLAX STRAW

The volume of gas produced by the seed flax straw was somewhat higher at the lower temperature. The rates of gas production were almost identical at the two temperatures for the first 8 days of digestion, after which there seemed to be no appreciable increase in gas at the higher temperature, a volume of 216 cc after 8 days increasing to only 231 cc after 20 days. Apparently some change took place which stopped fermentation after 8 days. At the lower temperature, however, the slow evolution of gas continued so that a volume of 232 cc after 8 days rose to 293 cc in 21 days and 317 cc in 34 days. The total amount of gas produced per gram of volatile solids added was very much smaller in the seed flax straw than in any of the other substances tested except lignin, being only 317 cc per gram (5.1 cubic feet per pound) after 34 days at 28° to 30° C. and only 231 cc per gram (3.7 cubic feet per pound) after 20 days at 50° to 55°. Of the total gas produced, 90 percent was evolved in about 16.5 days at the lower temperature, whereas at the higher temperature only about 6.5 days were required for the same proportion of gas. The time required for the evolution of 200 cc of gas per gram of volatile solids added was about 6.5 days at each of the two temperatures.

The quantity of methane present was 58.8 percent at 28° to 30° C. and 55.7 percent at 50° to 55°

DISCUSSION

In general, the materials tested fall into four distinct groups on the basis of the rate of gas production and the quantity of gas produced per gram of material added. This is very well shown in figure 2, particularly in *A*.

One group, which includes filter paper, artichoke-top flour, cornstalk flour, and cook liquor, is characterized by a high rate of gas production and a large volume of gas per unit of material added. Each of these substances produced 450 cc or more of gas per gram of volatile materials added (7.2 cubic feet per pound). At 28° to 30° C. each of these substances produced 200 cc of gas per gram (3.2 cubic feet per pound) of volatile material added in 6 days or less and 300 cc per gram (4.8 cubic feet per pound) in less than 8 days. At 50° to 55°, 400 cc or more of gas per gram of volatile solids added (6.4 cubic feet per pound) was produced in approximately 6 days or less, 300 cc (4.8 cubic feet per pound) in less than 4 days, and 200 cc (3.2 cubic feet per pound) in about 2 days.

The second group of materials includes chopped wheat straw, ground wheat straw, and chopped cornstalks. This group is characterized by moderate gas production (350 to 385 cc per gram of volatile material added at 28° to 30° C. and about 325 cc at 50° to 55°) and a rate of gas production distinctly lower than that in the first group. Thus at 28° to 30° it required 9 to 14 days for the production of 200 cc and 17 to 22 days for 300 cc of gas per gram of volatile solids added. At 50° to 55°, 200 cc of gas was produced in 3 to 5 days; 300 cc was produced in 11 days by the wheat staw and in 6 days by the cornstalks.

The seed flax straw is in a group by itself. The rate of gas production at the low temperature was intermediate between the first two groups, but the total volume of gas produced was distinctly less than that in either group. At the higher temperature the rate of gas production was about the same as that in the second group, but the total gas produced was very much less.

Lignin is also in a class by itself in that there is no evidence that it was decomposed with the production of gas, a phenomenon that has been previously reported by Levine et al. (*10*), Boruff and Buswell (*5*), and Acharya (*1*).

The question arises as to the reasons for the different rates of decomposition of the various substances tested, and whether these differences might be correlated with the chemical composition of the materials. The substances in the first group, characterized by a rapid rate of gas production and a large quantity of gas, (1) were entirely free from lignin (filter paper); or (2) had a high content of carbonaceous materials other than pentosans, cellulose, and lignin (cook liquor and artichoke-top flour); or (3) consisted of very small particles (cornstalk flour). The high rate of gas production and large volume of gas in the first three subtsances are therefore attributed to the comparatively large quantities of carbonaceous materials other than lignin and cellulose in the cook liquor and artichoke-top flour and to the homogeneous character and lack of lignin in the filter paper The rapid fermentation of the cornstalk flour (in contrast to the chopped cornstalks) is attributed to the small size of the particles and consequent large surface exposure.

The rate of decomposition of fibrous material subjected to anaerobic digestion is influenced by a number of factors, among which are temperature, size of particles subjected to decomposition, and chemical composition of the material.

A comparison of the results obtained with cornstalks and wheat straw indicates, however, that factors other than chemical composition (content of cellulose, lignin, and pentosans) and the size of particles are significant with respect to rate of decomposition at various temperatures. Table 3 shows the time required for the production of 300 cc of gas per gram of volatile matter added as chopped cornstalks, cornstalk flour, chopped wheat straw, and ground wheat straw.

TABLE 3.—*Time required for production of 300 cc of gas per gram of volatile matter in cornstalks and wheat straw*

Temperature (° C)	Cornstalks		Wheat straw	
	Chopped	Flour	Chopped	Ground
	Days	*Days*	*Days*	*Days*
28–30	22	7.5	25	16.5
50–55	6	3.5	11	11.5

It will be noted that the time required for the production of 300 cc of gas at 28° to 30° C. was approximately the same for the chopped cornstalks and the chopped wheat straw and that at 50° to 55° the digestion periods were almost identical for the chopped and for the ground wheat straw. In view of the fact that the chopped wheat straw was very much smaller than the chopped cornstalks and the ground wheat straw was smaller than the chopped wheat straw and considering further that the chemical composition of all these substances was practically the same, it is apparent that factors in addition to size and chemical composition must be concerned with the differences in rates of decomposition observed. If the rate of decomposition depended merely on size and chemical content, the smaller chopped wheat straw should have decomposed more readily than the chopped cornstalks at the lower temperature, and the finer ground wheat straw more rapidly than the chopped wheat straw at the higher temperature. A comparison of the chopped wheat straw and chopped cornstalks at the higher temperature, emphasizes further that size alone does not account for the differences observed in rates of decomposition. Here it will be noted that the chopped cornstalks required 6 days for the production of 300 cc of gas, while the chopped wheat straw, which was smaller and should therefore presumably have been attacked more readily if size alone were the determining factor, required 11 days. The effect of temperature on the rate of decomposition was appreciably different with the chopped cornstalks and chopped wheat straw. With the former the period for the production of 300 cc of gas dropped from 22 to 6 days as the temperature of digestion was raised from 28°–30° to 50°–55°; with the latter it dropped from 25 to 11 days.

It is suggested that the physical structure may mechanically protect the fermentable pentosans and celluloses of some materials against biological action. This concept might explain the difference in the rate of decomposition of different size particles of the same material. Another explanation is that the chemical combination of some fermentable substances prevents them from being readily available to micro-organisms. This suggestion might explain why the chopped cornstalks decomposed more rapidly than the wheat straw, which was distinctly smaller.

It will be noticed from figure 3 that the effect of temperature is much more marked on the chopped cornstalks than on the cornstalk flour. This indicates that fermentable substances which are mechanically protected from bacterial action are made more readily available to micro-organisms and their enzymes by the exposure resulting from comminution. Mechanical protection is presumably more effective at the lower temperature than at the higher temperature, where the rate of diffusion of enzymes is considerably greater.

In the experiments with seed flax straw no appreciable difference was observed in the rate of gas production as a result of the change in temperature. This material happened to be particularly high in cellulose though distinctly lower in pentosans than the cornstalks and wheat straw, but the volume of gas produced was markedly smaller than that produced by any of the other substances except lignin. The total pentosans and cellulose amounted to approximately 69 percent in the seed flax straw and approximately 62 percent in the cornstalks and wheat straw. The small amount of gas produced by the seed flax straw indicates that the fermentable substances were in some manner not readily available to microbial action. A considerable portion of this material consisted of shives, which are not readily fermentable, although analysis indicates that their content of cellulose, pentosans, and lignin does not differ appreciably from that in other materials. This is another indication that the content of these three constituents is not an index of the fermentability of a substance.

Apparently either mechanical protection or chemical linkage of the pentosans, cellulose, and other constituents markedly influences the digestibility and rate of decomposition of plant materials. Consequently, at present a fermentation test is the only available means for determining the susceptibility of a substance to microbial decomposition for the production of fuel gases.

The fact that the effect of temperature on the rate of digestion of different materials tested was not in all cases the same (in some instances gas production being three times as rapid at 50° to 55° C. as at 28° to 30°, while in others no significant differences were shown) emphasizes the importance of testing each material at various temperatures. The results obtained with one farm waste evidently cannot be applied to another as far as the effect of temperature on rate of anaerobic production of gas is concerned.

SUMMARY AND CONCLUSIONS

The rate and quantity of gas produced by the anaerobic decomposition of chopped cornstalks, chopped wheat straw, ground wheat straw, cornstalk flour, filter paper, seed flax straw, artichoke-top flour, cook liquor from wallboard manufacture, and alkali lignin were determined at 28° to 30° and 50° to 55° C.

The effect of temperature on the rate of anaerobic decomposition was different for the various fibrous farm wastes tested. Thus, with artichoke-top flour and seed flax straw, the rate of digestion at 28° to 30° C. was not appreciably different from that at 50° to 55°. With filter paper and cook liquor the same quantity of gas (300 cc per gram of volatile solids) was produced in about one-third the time at 50° to 55°. With cornstalk flour and chopped wheat straw it required approximately half as long to produce 300 cc of gas at 55° as at 28° to 30°. With ground wheat straw, the digestion time was reduced about one-third by employment of the higher temperatures, while with chopped cornstalks only about one-quarter of the digestion period was necessary at 50° to 55° as at 28° to 30°, to produce the same amount of gas. In view of the fact that the chemical composition of these substances is almost identical, it is apparent that composition cannot be used as a basis for predicting the probable influence of temperature on the rate of digestion.

The effect of temperature on the rate of digestion of fibrous farm wastes is more marked with coarser materials, such as chopped cornstalks and chopped wheat straw, than with finer materials, such as cornstalk flour and ground wheat straw.

The chemical composition of fibrous wastes (their content of lignin, cellulose, and pentosans) is not a dependable basis for evaluation of ease of anaerobic decomposition or of the rate and quantity of gas produced per unit weight of material.

Physical factors play an important role in the susceptibility of fibrous material to anaerobic microbial decomposition.

At present a fermentation test under controlled conditions is the only means of ascertaining the rate and quantity of gas obtainable from a fibrous farm waste by anaerobic microbial decomposition.

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INACTIVATION OF PYRETHRUM AFTER INGESTION BY THE SOUTHERN ARMYWORM AND DURING INCUBATION WITH ITS TISSUES[1]

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INTRODUCTION

Pyrethrum is widely used as a contact insecticide, but it has not been found generally effective as a stomach poison. In experiments with larvae of the southern armyworm (*Prodenia eridania* (Cram.)) the writer has found that, although they are killed by pyrethrum applied externally, they are apparently not affected after ingestion of the substance. Furthermore, the feces of these larvae failed to show the presence of the toxic principles of pyrethrum when tested against mosquito larvae. The purpose of this investigation was to determine whether the toxic principles of pyrethrum are inactivated, in whole or in part, after ingestion by the southern armyworm, and the kind of tissues or the conditions that might be responsible for such inactivation.

Swingle (*10*)[2] found that, although larvae of the imported cabbage worm (*Pieris rapae* L.) are rapidly affected and killed by pyrethrum applied externally, they are not killed after ingestion of the substance. He further demonstrated[3] that, even though the larvae appear to suffer no ill effects, the toxic principles do not pass through the insect unchanged, for feces of larvae that had ingested pyrethrum were not toxic when applied as a contact dust to other larvae of the same species. Swingle suggested that the action of the digestive juices apparently had destroyed the toxicity

Audiffren (*3*) obtained a chemical reaction indicating the presence of chrysanthemum monocarboxylic acid in the urine of human patients to whom pyrethrum had been administered as an anthelmintic. This indicates the cleavage of pyrethrin I.

MATERIALS

The larvae used in these experiments were sixth instars of the southern armyworm, reared in a greenhouse insectary on turnip plants and cut lettuce.

The unextracted pyrethrum used was powdered Kenya pyrethrum flowers having a pyrethrin I content of 0.53 percent and a pyrethrin II content of 0.64 percent, as determined by the Seil method.[4] A petroleum ether extract of pyrethrum was also used in one experiment.

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[1] Received for publication August 3, 1938

[2] Italic numbers in parentheses refer to Literature Cited, p. 294.

[3] Unpublished report, 1934

[4] This analysis was made by S I. Gertler, of the Division of Insecticide Investigations of this Bureau.

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Toxicity was determined by the use of mosquito larvae (*Culex fatigans* Wied.) as biological indicators, according to the method reported by Campbell and associates (*5*, *9*). This method has been used successfully for the determination of comparative toxicity of a number of insecticidal substances, including pyrethrum, to which mosquito larvae are very sensitive.

INACTIVATION AFTER INGESTION BY SOUTHERN ARMYWORM LARVAE

Since there is evidence (*8*, *14*) that the toxic principles may be absorbed directly through the body integument, it appears not unlikely that they could be absorbed through the gut wall. The fact that southern armyworm larvae are unaffected after ingestion of pyrethrum, and that the feces are nontoxic to mosquito larvae, indicates that the toxicity has been destroyed either within the gut or within the tissues after absorption through the gut wall.

PROCEDURE

In this experiment southern armyworm larvae were fed pyrethrum, and later all the tissues of the larvae as well as the gut contents and feces were tested against mosquito larvae for the presence of the toxic principles.

The pyrethrum was fed to the larvae in sandwiches prepared according to the method of Campbell and Filmer (*4*). Disks 22 mm in diameter were cut from turnip leaves and dusted with pyrethrum by the air-float method under a large battery jar. Each disk received approximately 1 mg of the dust. The sandwiches were then prepared, starch paste being used as the adhesive. Each sandwich was placed in a 150-ml beaker with one larva that had been deprived of food for several hours and retained there for 18 hours overnight. In the morning those larvae that had ingested entire sandwiches and had not regurgitated more than a negligible amount were conserved on fresh turnip leaves for 6 hours longer. All the feces excreted during the 24-hour period, and also the washings of the beakers in which the larvae had been conserved, were collected and combined. The blood of each larva was obtained by snipping an abdominal leg, and the blood of all the larvae was combined. The larvae were then dissected, the following different kinds of materials were isolated, and like materials from all the larvae were combined and ground with sand: Skin, tissue of the digestive tract and the malpighian tubules, fat body, muscular and all other tissues, contents of the foregut and hind-gut, and contents of the midgut. The different kinds of materials prepared as outlined above from larvae that had ingested pyrethrum will be referred to as tests to distinguish them from the checks, which were prepared at the same time.

Three checks were used. The first was designed to demonstrate the effect of the larval tissues alone on the mortality of the mosquito larvae, the second the combined effects of larval tissue and pyrethrum, and the third the effect of pyrethrum alone. For the first and second checks the tissue preparations were made from larvae that had received the same treatment as those used for the tests except that the sandwiches ingested did not contain pyrethrum. For the second check pyrethrum was mixed with the tissues while they were being ground. For the third check microscope cover glasses of the same diameter as the leaf disks were dusted with pyrethrum when the leaf disks were dusted, so that the quantity of pyrethrum would be the same as that ingested by the larvae.

The tests and checks were diluted with distilled water. The exact concentration of the pyrethrum in the suspensions as prepared is unknown, as the quantity of dust on the leaf disks could not be determined with accuracy. However, the exact strength is immaterial to the present investigation. The pyrethrum-only check was prepared in such concentration that it gave 100-percent morbidity or mortality of the mosquito larvae within 1 hour. The proper concentration to use was obtained by preliminary trials. The check consisting of larval materials to which pyrethrum had been added was prepared in the same concentration. The tests and the checks consisting of the various larval materials without pyrethrum were prepared 100 times as concentrated as the pyrethrum-only check. The morbidity or mortality of the mosquito larvae in so strong a concentration should indicate the presence of even greatly reduced quantities of the toxic principles in the materials from larvae that had ingested pyrethrum.

One hundred mosquito larvae were used in each concentration of each test and check. The mortality was determined after 1 hour and after 20 hours at 29° C. in the dark.

RESULTS

All the mosquito larvae in the pyrethrum-only check and in the pyrethrum-added check were dead or moribund within 1 hour. In the tests and in the no-pyrethrum check the mortality was negligible even though the concentration was 100 times that of the pyrethrum-only check and the period of exposure was 20 hours. Similar results were obtained on two repetitions.

The results of this experiment indicate that 6 to 24 hours after ingestion little, if any, of the toxic principles of pyrethrum are present in any of the tissues of larvae that have ingested pyrethrum, in the contents of the digestive tract, or in the feces. It is concluded, therefore, that the toxic principles of pyrethrum are inactivated, either wholly or in high degree, after ingestion by the southern armyworm.

INACTIVATION BY THE ISOLATED TISSUES OF SOUTHERN ARMYWORM LARVAE

To identify the tissues responsible for any possible inactivation of the toxic principles of pyrethrum, an experiment was made to determine whether the toxicity would be destroyed or altered by incubation with one or more of the isolated tissues.

Since the pyrethrins occur chiefly within the cell walls in the achenes of pyrethrum flowers, they are not immediately exposed to the action of their environment even in fine powder. A slight reduction in toxicity of unextracted pyrethrum powder that was noted in unreported preliminary experiments might have been due to the breakdown of only that small portion of the pyrethrins that was liberated in grinding. A petroleum ether extract of pyrethrum was therefore used in addition to the unextracted pyrethrum.

PROCEDURE

The blood, fat body, skin and muscle, digestive tract, and gut contents from healthy larvae that had not ingested pyrethrum were isolated, and like tissues were combined and ground with sand. In one series of tests unextracted pyrethrum suspended in distilled water in the proportion of 1 mg per milliliter was added at the rate of 0.5 mg per larva, and in another series a petroleum ether extract of pyrethrum suspended in distilled water in the proportion of 0.16 mg per milliliter was added at the rate of 0.08 mg per larva.

Two checks were used. The first check was designed to demonstrate the effects of the larval tissues alone on the mortality of the mosquito larvae, and was made with tissue preparations corresponding to those used in the tests, to which water, not containing pyrethrum, but equivalent in amount to the pyrethrum suspension, was added. The purpose of the second check was to demonstrate the effect of pyrethrum alone, and was made with an amount of pyrethrum equivalent to that mixed with the tissues in the tests, suspended in water.

The preparations were incubated in the dark for 18 hours at 29° C. The material used in each series was divided into two parts. One part was allowed to stand in shallow vessels during the incubation. Since unreported experiments had demonstrated that a considerable quantity of the toxic principles settles out of suspension during this period, resulting in their unequal distribution in the tissue preparations, the other part was agitated in a mechanical shaker throughout the incubation period.

Toxicity was determined by the use of mosquito larvae as described for the first experiment. The pyrethrum-only check was prepared in such concentration that it gave 100-percent morbidity or mortality of mosquito larvae within 2 hours. Concentrations one-fifth, one twenty-fifth, and one-fiftieth this strength were also prepared. The tests and the no-pyrethrum check were prepared in the same concentrations.

RESULTS

The effect of the larval materials alone on the mortality of the mosquito larvae in the tests may be disregarded, since the mortality in the checks containing larval material but no pyrethrum was negligible even after 20 hours. With both unextracted and extracted pyrethrum the mosquito larvae in the checks containing highly concentrated pyrethrum were dead or moribund within 2 hours. In the lowest concentration the mortality was low even after 20 hours. The degree of reduction in toxicity of the toxic principles of both the unextracted and the extracted pyrethrum during incubation with various tissues and with the gut contents of southern armyworm larvae was estimated by comparing the mortality of the mosquito larvae in these tests with that in the pyrethrum check that was incubated in water without larval materials. The results are given in table 1.

TABLE 1.—*Reduction in toxicity of unextracted and extracted pyrethrum during incubation with various tissues and with the gut contents of southern armyworm larvae, as indicated by the mortality of mosquito larvae*

Tissue	Degree of reduction in toxicity			
	Not agitated		Agitated	
	Unextracted	Extracted	Unextracted	Extracted
Blood	Little or none	Little or none	Little or none	Partial?
Fat body	Partial	Great	Very great	Very great.
Skin and muscle	do	Partial	Partial	Great
Digestive tract	Little or none	do	Little or none	Partial.
Gut contents	do	do	do	Partial?

The extracted pyrethrum underwent slightly greater reduction in toxicity than the unextracted material, and agitation throughout the incubation period increased the reduction in toxicity of both extracted and unextracted pyrethrum.

With the possible exception of the agitated extracted material, neither extracted nor unextracted material was appreciably reduced in toxicity by incubation with blood. The toxicity of unextracted pyrethrum was not appreciably reduced by incubation with either the tissue or the contents of the digestive tract. The toxicity of extracted pyrethrum was partly reduced by incubation with these materials, but the degree of reduction was not increased by agitation. Both extracted and unextracted pyrethrum were reduced in toxicity by incubation with skin and muscle tissue, and the reduction of extracted pyrethrum was increased by agitation. The toxicity of unextracted pyrethrum was partly reduced, and that of extracted pyrethrum greatly reduced, when incubated with fat body and not agitated. Very great reduction occurred with both extracted and unextracted material when agitated during incubation.

DISCUSSION

The high degree of inactivation of pyrethrum which has been shown to occur after ingestion by the southern armyworm and during exposure to different tissues of this insect may be explained by the fact that the pyrethrins I and II, the toxic principles of pyrethrum, are unsaturated keto esters and chemically unstable. A great variety of chemical and physical conditions exist in the insect and in insect tissues that, singly or combined, might influence the break-down of the pyrethrins. The possible effect of hydrolytic enzymes, such as esterases, on the decomposition of the pyrethrins has been suggested (*2*).

It is well known that pyrethrum may lose insecticidal value in storage and that it is affected by heat, moisture, light, and other natural conditions (*1*, *6*, *7*, *13*, *15*). Tattersfield (*11*) and Tattersfield and Martin (*13*) have shown that pyrethrum powders and dusts lose their insecticidal activity on exposure to light and air, and that such loss of activity is retarded by the incorporation of certain antioxidants with the pyrethrum-dust preparations. Tattersfield and Hobson (*12*) found pyrethrum extracts to be comparatively stable in solution or suspension in certain solvents in closed vessels and in the presence of various emulsifiers.

The decrease in toxicity of pyrethrum during incubation with certain tissues of armyworm larvae suggests that some of these tissues or their products may be responsible for the inactivation which occurs in living larvae. Since the two conditions are quite different, no definite conclusion can be drawn. It is doubtful that the tissues or secretions of the digestive tract are alone responsible. The fat body alone probably could inactivate the pyrethrins after their liberation from the cell walls in the achenes and their absorption into the hemolymph.

SUMMARY AND CONCLUSIONS

An investigation was undertaken to determine whether the toxic principles of pyrethrum are inactivated, in whole or in part, after ingestion by the southern armyworm (*Prodenia eridania* (Cram.)), and also to learn the kind of tissues that might be responsible for such inactivation. To study the first problem the pyrethrum was fed to the larvae in turnip-leaf sandwiches, and later the tissues, gut contents, and feces were ground and tested against mosquito larvae for the presence of toxic principles. To study the second problem the pyrethrum was mixed with the various tissues and the gut contents from healthy larvae and incubated at 29° C. in the dark for 18 hours. Half the material was agitated during incubation. The material was then tested against mosquito larvae for the presence of toxic principles.

The results indicate that the toxic principles of pyrethrum may become inactivated, wholly or in high degree, after ingestion by the southern armyworm. The tissue of the digestive tract and the gut contents may reduce the toxicity of unextracted pyrethrum little or none, and of extracted pyrethrum only partially. The blood may have little or no effect on the toxicity. The skin and muscle tissues may greatly reduce the toxicity of agitated extracted pyrethrum, but only partially reduce the toxicity of unagitated extracted pyrethrum and of either unagitated or agitated unextracted pyrethrum. The fat body may reduce the toxicity of unextracted pyrethrum partially and of extracted pyrethrum greatly, even when these materials are not agitated, but the toxicity of both when agitated with fat body during incubation may be very greatly reduced.

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CYTOGENETIC OBSERVATIONS IN LACTUCA[1]

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INTRODUCTION

The work on *Lactuca* reported in this paper was initiated with a twofold purpose: (1) To make a survey of meiosis in certain species and (2) to define the limits of interspecific hybridization in order to determine the possibility of introducing desirable genes of other species into cultivated lettuce (*Lactuca sativa* L.).

REVIEW OF LITERATURE

Recently Babcock, Stebbins, and Jenkins (*1*) [3] have reviewed the pertinent literature. These authors have reported the chromosome numbers of 25 species of *Lactuca*. Of the species of *Lactuca* the chromosome number of which is known, 11 have 8 pairs, 12 have 9 pairs, and 6 are presumably amphidiploids with 17 pairs of chromosomes. The latter are endemic American species and are thought to have originated from crosses between species with eight and nine chromosomes, followed by doubling of the entire complement.

A paper by Cooper and Mahony (*2*) is also of interest. In a study of meiosis in *Lactuca scariola* var. *integrata*, they found that the plants examined were structural hybrids with seven bivalents and a ring of four chromosomes. Data on fertility and condition of the pollen were not given, hence there is no means of judging the physiological effects of the translocation. In earlier work by Gates and Rees (*5*) with *L. sativa* 50 percent of the first metaphase plates showed less than the characteristic haploid number of nine chromosomes. Thus it appears likely that they also were dealing with plants in which translocation had occurred.

There are some reports of attempted species crossing in the genus. Ernst-Schwarzenbach (*4*) made reciprocal pollinations, using *Lactuca scariola* and a number of cultivated varieties of *L. sativa*. The majority of these pollinations were successful and resulted in viable seed. A few reciprocal pollinations were made between *L. scariola* and *L. virosa;* none set seed. Negative results were obtained in 12 further crossings in which *L. virosa* was used as a pollen and seed parent with 10 varieties of *L. sativa.*

Durst (*3*) has crossed several varieties of *Lactuca sativa* with *L. scariola*. From data secured in F_2 and subsequent generations he concluded that differences between the two species are largely accounted for by mutations of dominant genes.

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[1] Received for publication August 19, 1938.

[2] The authors gratefully acknowledge the assistance of the Division of Plant Exploration and Introduction in supplying much of the material for the investigation here reported. Thanks are also extended to Dr. G. L. Stebbins, Jr., Division of Genetics, University of California, for identifying the material.

[3] Italic numbers in parentheses refer to Literature Cited, p. 306.

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MATERIALS AND METHODS

The species of *Lactuca* studied by the authors were assembled at the United States Horticultural Field Station, La Jolla, Calif. The following is a list of the species and varieties, with the sources from which they were obtained:

Lactuca sativa var. Imperial D	Jagger.
Lactuca sativa var. Chinese Stem	P. E. and I.,[1] from China.
Lactuca sativa var. Bavarian Brown	P. E. and I., from Europe.
Lactuca sativa	P. E. and I., from India.
Lactuca scariola var. *integrata* Gren. and Godr.	Collected at Woodland, Calif.
Lactuca scariola L.	P. E. and I., from Union of Soviet Socialist Republics.
Lactuca scariola var. *angustana* Hort.	P. E. and I., from Sweden.
F_1 (Imperial 152 × *L. scariola*)	Jagger.
Lactuca chondrillaeflora Bor.	P. E. and I., from France.
Lactuca perennis L.	P. E. and I., from England.
Lactuca tartarica (L.) C. A. Meyer	P. E. and I., from India.
Lactuca cretica Desf.	P. E. and I., from Union of Soviet Socialist Republics
Lactuca bourgaei Boiss.	P. E. and I., from England.
Lactuca canadensis L.	J. B. Norton, Hartsville, S. C.
Lactuca graminifolia Mich.	Do.

The cytological observations were for the most part made on acetocarmine smears of meiotic chromosomes. In some cases the counts were checked by root-tip studies.

The preparation of the slides of *Lactuca* material was rather difficult, and it was found necessary to develop a special technique for their preservation as permanent smears. Since the technique differs in several ways from the ordinary methods of permanent smear preparation, it will be described here in some detail. Flower heads in which the pollen mother cells are actively dividing are found by making a temporary acetocarmine smear of an individual floret. The remaining florets are fixed in a mixture of acetic acid and absolute alcohol (1 part glacial acetic acid, 2 parts absolute alcohol). They are allowed to remain in this mixture for 2 to 3 hours. The fixative is then drained off and replaced with absolute alcohol, in which the material is permitted to stand overnight. This procedure seems to harden the delicate pollen mother cells; consequently they are not easily crushed in the smearing process. The individual flowers are placed on a slide in a drop of acetocarmine, and the anthers are dissected out and crushed. The extraneous material is removed, and the cover slip added. By heating gently, and with slight pressure on the cover slip, it is possible to cause the cells to adhere either to the slide or to the cover slip. Heating also promotes greater differentiation between chromosomes and cytoplasm.

The cover is floated off in a mixture of equal parts of absolute alcohol and acetic acid. Both slide and cover are transferred through several changes of 95-percent alcohol and allowed to remain 15 minutes or longer in the latter solution. Slide and cover slip are then reunited by mounting in either euparal or diaphane. This step should be carried out in an atmosphere of low humidity, and care should be exercised to avoid breathing on the slide lest the preparation become fogged and unsuitable for observation. By mounting directly in euparal or diaphane, immersion in the higher alcohols and xylols becomes unnecessary. This is a point of considerable importance, since the latter two fluids, especially xylol, are prone to distort and darken the cytoplasm. Material prepared according to the above schedule has proved very satisfactory. Such preparations are particularly suitable for purposes of photomicrography, since the cells are flattened and the entire chromosome complement is brought into one plane.

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[1] Division of Plant Exploration and Introduction, Bureau of Plant Industry, U. S. Department of Agriculture.

In the hybridization work, the usual "depollination" method of Oliver (*7*) has been followed. This consists in washing the pollen from the expanded stigmas by means of a fine stream of water. The stigmas are then dried and dusted with pollen from the desired plants. Protection from insect contamination after pollination seems to be unnecessary.

OBSERVATIONS

The information obtained as to chromosome number, morphology of chromosome complement, and pollen fertility has been recorded in table 1. The letters L, M, and S refer to the relative lengths of the chromosomes within a genom. The percentage of pollen fertility was determined by counts from acetocarmine smears of mature anthers.

MEIOSIS IN THE NINE-CHROMOSOME SPECIES

In all varieties of *Lactuca sativa* the nine bivalents pair regularly (fig. 1, *A*). Three are definitely longer than the remaining members of the complement, and three are much shorter; thus the relationship may be expressed by the ideogram 3L+3M+3S. This seems to be valid for all varieties of *L. sativa* examined. An occasional irregularity may occur when plants are subjected to abnormally high temperatures during meiosis. Such a case is shown in figure 1, *B*, where one bivalent has separated somewhat in advance of the others. It is doubtful whether a disturbance of this nature would cause the production of sterile pollen, and it must be concluded that in its chromosome behavior *L. sativa* is a very stable species.

TABLE 1.—*Chromosome number, ideogram, and pollen fertility in species of Lactuca*

Species	Chromosomes	Ideogram [1]	Pollen fertility
	Number		*Percent*
Lactuca sativa var. Imperial D	9	3L+3M+3S	90-95
L. sativa var. Chinese Stem	9	3L+3M+3S	90
L. sativa var. Bavarian Brown	9	3L+3M+3S	90
L. sativa (from India)	9		90-95
L. scariola var. *integrata* (from California)	9	4L+2M+3S	80-85
L. scariola (from Union of Soviet Socialist Republics)	9	4L+2M+3S	90
L. scariola var. *angustana*	9	4L+2M+3S	90
F_1 (Imperial 152 × *L. scariola*)	9	3L+3M+3S	90
L. chondrillaeflora	9	2L+7M	85-90
L. perennis	9	7M+2S	90
L. tartarica	9	3L+6M	90
L. cretica	8	4L+4M	90-95
L. bourgaei	8	8M	80-85
L. canadensis	17		90-92
L. graminifolia	17		85-90

[1] Relative length of chromosomes is indicated by the initial letters of the words "long," "medium," and "short."

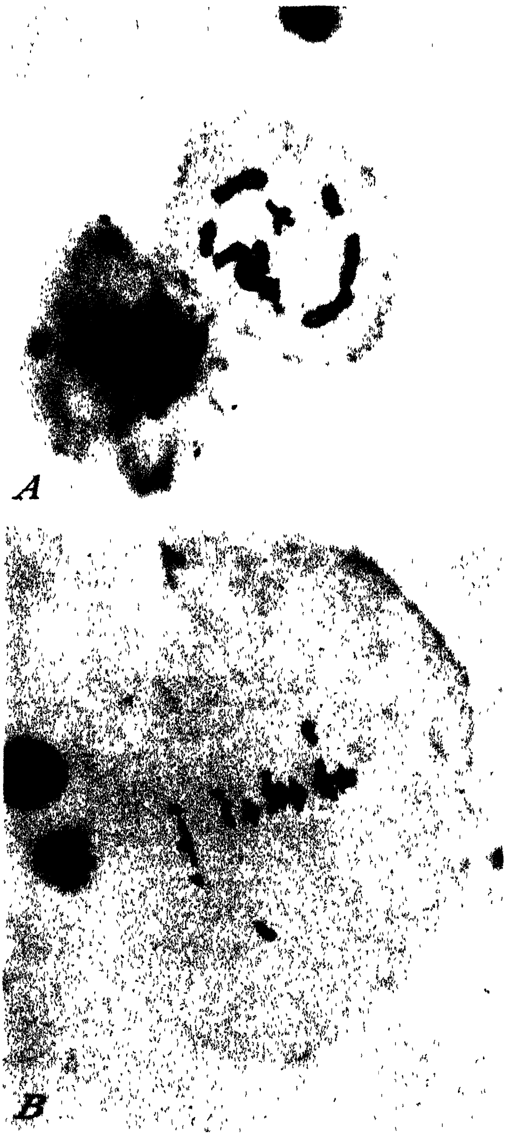

FIGURE 1.—Photomicrographs showing chromosomes of cultivated lettuce (*Lactuca sativa*): *A*, *L. sativa* var. Imperial D, diakinesis (× 1,100); *B*, *L. sativa* var. Imperial D, metaphase, profile view, showing one bivalent dividing precociously (× 820).

The nine chromosomes of *Lactuca scariola* are morphologically very similar to those of *L. sativa* (fig. 2, *A*). A close study, however, indicates that there is not the extreme differentiation in size of the individual chromosomes within the set that is found in *L. sativa*. There seem to be four bivalents of somewhat greater length than the remainder, and three that are somewhat shorter. The complement of *L. scariola* may therefore be represented by the ideogram 4L+2M+3S. A number of strains and varieties of *L. scariola* from widely scattered geographic ranges have been examined and no differences in the morphology of the chromosome complement have been found.

In spite of possible slight differences in the make-up of their genoms, fertile F_1 hybrids in which the chromosomes pair regularly at meiosis (fig. 2, *B*) can be obtained easily between varieties of *Lactuca scariola* and *L. sativa*. The percentage of viable pollen is approximately the same as in the parent species. There are some indications of hybrid vigor in the F_1, but these are not striking. The fact that varieties of *L. sativa* and *L. scariola* can be crossed at will, with the production of fertile offspring, is of very real importance. Strains that are resistant to mildew (*Bremia* sp.) and that promise to become commercially acceptable varieties have been selected from original crosses of this type.

Lactuca chondrillaeflora.—This species has inconspicuous yellow flowers, and the unusual habit of shedding its leaves with the initiation of the flowering cycle. There are nine chromosomes, two of which are definitely longer than the remainder (fig. 2, *C*). Meiosis is regular, and pollen fertility high (85 to 90 percent). Attempts to hybridize this species with *L. sativa* and the tetraploids *L. canadensis* and *L. graminifolia* were unsuccessful (table 2).

TABLE 2.—*Summary of hybridization experiments with Lactuca species* [1]

Species involved	Results	Cytological condition of F_1
L. sativa × *L. scariola*	Fertile seed	Regular pairing
L. sativa × *L. chondrillaeflora*	No seed	
L. sativa × *L. perennis*	do	
L. sativa × *L. canadensis*	do	
L. sativa × *L. graminifolia*	do	
L. sativa × *L. cretica*	do	
L. scariola × *L. canadensis*	do	
L. scariola × *L. graminifolia*	do	
L. graminifolia × *L. canadensis*	Fertile seed	Regular pairing

[1] In all of the cases listed, at least 6 flower heads were pollinated. Reciprocal pollinations were made except in the case of *L. sativa* × *L. chondrillaeflora*.

Lactuca perennis.—Of the nine chromosomes of this species, two are markedly shorter than the rest. All of the bivalents are in the form of rings or chains at diakinesis (fig. 2, *D*). Plants of *L. perennis* have not produced seed, yet there is a high percentage of pollen fertility (90 percent). In this case the inability to produce viable seed is evidently not conditioned by chromosomal irregularities. The chromosome complement of this species differs in several respects from that of *L. sativa*: (1) In morphology; (2) in method of pairing, i. e., in *L. perennis* the bivalents are either in rings or chains; and (3) in number of chiasmata per bivalent (*L. perennis*, 1.5; *L. sativa*, 2.0).

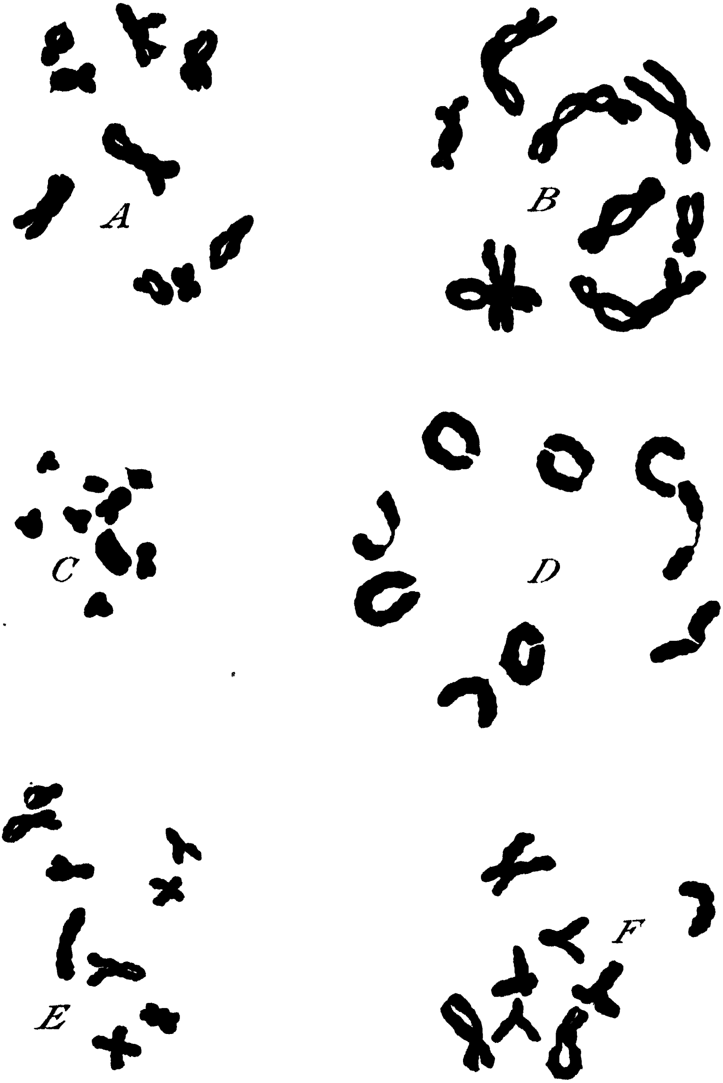

FIGURE 2.—Chromosomes from pollen mother cells of *Lactuca* species (× 2,000): *A*, *L. scariola*, diakinesis; *B*, F_1 (Imperial 152 × *L. scariola*), diakinesis, showing perfect pairing of all bivalents; *C*, *L. chondrillaeflora*, metaphase: *D*, *L. perennis*, diakinesis; *E*, *L. tartarica*, diakinesis; *F*, *L. cretica*, diakinesis.

Attempts have been made to cross *L. perennis* with *L. sativa.* Reciprocal matings were made in which two strains of *L. sativa* (Imperial 152 and Imperial D) were used. The results were negative.

Lactuca tartarica.—The chromosome complement of this blue-flowered *Lactuca* is comparable in many respects to that of *L. sativa,* or *L. scariola.* It has nine meiotic chromosomes, of which at least three are considerably longer than the rest (fig. 2, *E*).

MEIOSIS IN THE EIGHT-CHROMOSOME SPECIES

Lactuca cretica.—This large-flowered annual *Lactuca* has eight chromosomes at meiosis; four of the bivalents are of about equal size and somewhat longer than the remainder (fig. 2, *F*). Attempts have been made to cross this species with the nine-chromosome *L. sativa,* and the tetraploid, *L. canadensis,* without success (table 2). The writers have not been able to effect hybridization of *L. cretica* with the other eight-chromosome species, *L. bourgaei. L. cretica* flowers early in the spring and dies, whereas *L. bourgaei* comes into flower in late summer or early fall.

Lactuca bourgaei.—This striking perennial, blue-flowered *Lactuca* has eight bivalents of approximately equal length (fig 3, *A*). Pollen fertility (80 to 85 percent) is not quite as high as in the majority of the species of the genus. This amount of sterility is probably the result of the irregularities found during meiosis. The most common type, the formation of chromatin bridges, is illustrated in figure 3, *B*.

MEIOSIS IN THE 17-CHROMOSOME SPECIES

Lactuca canadensis and *L. graminifolia* [4] have 17 chromosomes at meiosis (fig. 3, *C* and *D*). There is considerable similarity in the chromosome complement of the two species. A study of meiosis in both species indicates that the chromosomes are paired as bivalents. Occasional multivalent associations have been noted, but these are comparatively rare. Pollen fertility in these species ranges from 80 to 95 percent, as shown in table 1. In the F_1 hybrid, pairing is regular (fig. 3, *E*). The percentage of pollen sterility and the production of viable seed indicate no loss of fertility.

The writers have had under observation at least 100 F_2 plants, and all of them appear to be normal. As a result of cytological observations the writers can state that the formation of multivalents is rather rare but that in some cases it is greater than that found in the parent species. In table 3 are summarized the cytogenetic observations of the F_1 and F_2 of the cross *Lactuca canadensis* × *L. graminifolia.*

TABLE 3.—*Cytogenetic observations of the progeny from the cross L. canadensis* × *L. graminifolia*

Progeny	Meiotic behavior	Viable pollen	Seed		Vigor
			Production	Germination	
		Percent			
F_1	Normal	85-90	Abundant	Good	Equal to either parent
F_2	do [1]	85 90	do	do	Equal to or surpassing both parental species

[1] In some cases, quite a number of multivalents

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[4] These two species were crossed at the suggestion of J. B Norton In the vicinity of Hartsville, S C, Mr. Norton has observed plants that appear to be natural hybrids of these two species

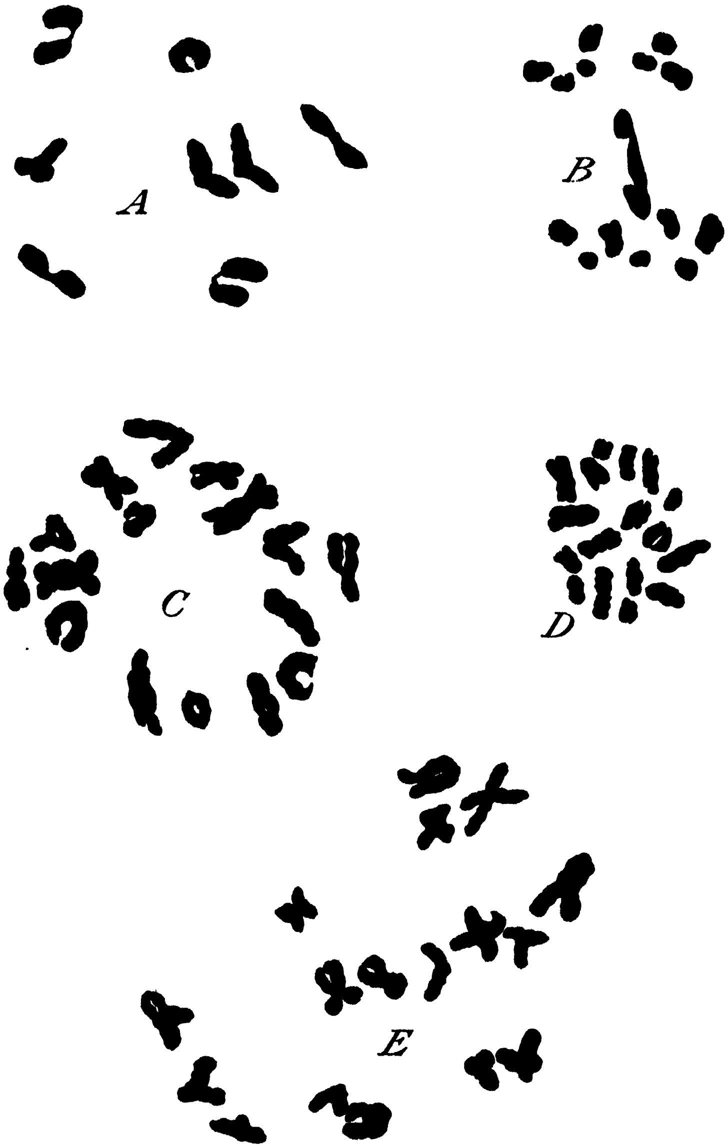

FIGURE 3.—Chromosomes from pollen mother cells of *Lactuca* species (× 2,000): *A*, *L. bourgaei*, diakinesis; *B*, *L. bourgaei*, late anaphase, showing bridge configuration; *C*, *L. canadensis*, diakinesis; *D*, *L. graminifolia*, early metaphase; *E*, F$_1$ (*L. canadensis* × *L. graminifolia*), diakinesis.

DISCUSSION

Results here reported from interspecific hybridization experiments in *Lactuca*, though not extensive, support the following tentative conclusions: (1) Only a single cross (*L. sativa* × *L. scariola*) was obtained within the group having nine chromosomes; (2) no hybrids were secured between species with nine chromosomes and those with eight chromosomes; (3) pollination of the tetraploids (amphidiploids) with pollen from both eight- and nine-chromosome groups gave negative results; (4) hybrids between the two tetraploids can be obtained easily; and (5) in the two cases where interspecific hybrids were obtained (*L. sativa* × *L. scariola* and *L. canadensis* × *L. graminifolia*), the progeny has been fertile and cytologically stable.

According to Lewitsky (*6*), the more primitive species of a group generally have chromosome complements with individuals of equal sizes and as advances are made toward a greater degree of specialization the individuals of a genom become more and more unequal in size. The size differences are presumed to occur as a result of unequal translocation. If the species of *Lactuca* with nine chromosomes are arranged according to this hypothesis, the varieties of *L. sativa* will occupy the most advanced position, with *L. scariola* next and *L. tartarica*, *L. chondrillaeflora*, and *L. perennis* in about that order.

Cooper and Mahony (*2*) found seven bivalents and a ring of four chromosomes in plants of *Lactuca scariola* var. *integrata*. The writers have been unable to confirm this finding, although they have examined collections from a number of stations in the United States and Europe. Their observations indicate that *L. scariola* is a highly variable species, both in morphological characters and in ability to adapt itself to various ecological situations. It therefore seems quite probable that further investigations may reveal strains with translocations.

It has been demonstrated that desirable genes of *Lactuca scariola* can be introduced into *L. sativa*. Plants with a high degree of fertility and cytologically stable are recovered in subsequent generations. This raises the question whether *L. sativa* can be considered as a species distinct from *L. scariola*. The following evidence indicates very definitely that the relationship is extremely close: (1) They cross readily, with the production of fertile seed; (2) F_1 and F_2 individuals are fertile (regular pairing in F_1); (3) parallel morphological variations found in varieties of *L. sativa* can be detected in collections of *L. scariola*, with the possible exception of variations toward the "heading" type.

The possibilities of introducing favorable genes into cultivated lettuce have not been exhausted. There are a number of other species of *Lactuca*, more or less closely related to *L. sativa*, that may be sources of such genes, and efforts should be made to find whether successful crosses can be obtained. In this respect *L. tartarica*, a species with nine chromosomes, may have potentialities; it is a strong, vigorous, rapidly growing perennial. These qualities if introduced into *L. sativa* might be utilized to good advantage.

The work of Babcock, Stebbins, and Jenkins (*1*) indicates that it may prove profitable to initiate hybridization experiments between certain of the eight- and nine-chromosome species that are thought to be involved in the origin of the amphidiploid American species. Such hybrids should shed some light on the origin of these species and on the puzzling problem of their relationship to each other.

SUMMARY

A technique is described for the preparation of permanent smears of *Lactuca* material.

A brief description of meiosis in the following species of *Lactuca* is given: *L. sativa*, *L. scariola*, *L. chondrillaeflora*, *L. perennis*, *L. tartarica*, *L. cretica*, *L. bourgaei*, *L. graminifolia*, and *L. canadensis*.

Reciprocal matings involving most of the above species have been tried. Fertile F_1 hybrids that have been obtained are *L. sativa* × *L. scariola* and *L. canadensis* × *L. graminifolia*. The F_1 and F_2 individuals are fertile and no cytological irregularities have been found.

The chromosome ideogram is given for all of the diploid species. The greatest degree of morphological differentiation of the chromosomes was found to occur in varieties of *L. sativa*.

With one exception (*Lactuca perennis*), the species under observation were self-fertile and had a high percentage of pollen fertility. In *L. bourgaei*, an eight-chromosome species the pollen fertility was somewhat reduced (80 to 85 percent), apparently as a result of irregularities during meiosis.

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BREED DIFFERENCES IN RESISTANCE TO A DEFICIENCY OF VITAMIN B_1 IN THE FOWL[1]

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INTRODUCTION

The breeds and varieties of domestic fowls differ in size and shape of body, in structure and color of plumage and skin, in variation of the skeleton, and in the modification of structures, such as the comb and spurs, arising from the skin. Apart from colors, the differentiation of the breeds at the time of their establishment was apparently based upon morphological characters. A few differences between breeds in physiological characters, such as broodiness and color of egg, are now known, but it is not impossible that the present comparative freedom of Leghorns from broodiness and the ability of the same breed to lay white-shelled eggs may have resulted largely from artificial selection for those physiological characters as for other distinguishing breed characteristics. This paper reports an association between breed characteristics and an interesting physiological character—resistance to a deficiency of vitamin B_1.

The first indication that White Leghorns require comparatively little vitamin B_1, or through differential storage of it, or by other means, are markedly resistant to polyneuritis, was put forward by Nichita and Iftimesco (*9*).[2] On a diet deficient in vitamin B_1, or lacking it entirely, one of three White Leghorns, 10 months old at the start of the experiment, showed symptoms of polyneuritis after 41 days and died in 49 days. Another was entirely unaffected and was removed from the experiment at 78 days. A third showed no symptoms till the one hundred and seventh day and for 3 weeks thereafter maintained a subacute chronic state of polyneuritis, but quickly recovered when placed on a normal diet. On the other hand, Nichita, Tuschak, and Calcef (*10*) found that on diets deficient in vitamin B_1 six Rhode Island Reds about 10 months old showed symptoms of polyneuritis at from 7 to 19 days. Three of them were given yeast and quickly recovered, but the other three died of acute polyneuritis in 9, 18, and 21 days. In both experiments, controls on the experimental diet plus yeast were entirely normal.

The experiments of Nichita and his coworkers were not set up primarily to test the two breeds as to their requirements of vitamin B_1, and unfortunately the diets used differed considerably. The diet of the Rhode Island Reds included fresh beef, glucose, and starch, whereas that the White Leghorns did not. The latter received their carbohydrates from decorticated rice. Hence, while the experiments within each breed were well controlled, the fact that the diets were not identical makes any comparison of the two breeds with respect to their

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requirement of vitamin B_1 less accurate than might be desired. Accordingly, experiments were planned by the present writers to determine in chicks of these two breeds their resistance to a deficiency of vitamin B_1. The results are reported in this paper.

MATERIALS AND METHODS

In four separate trials Single Comb White Leghorn chicks were compared with Rhode Island Red chicks of the same age upon a diet deficient in vitamin B_1. Barred Plymouth Rocks were also included in the fourth trial. Environmental conditions were made identical for all chicks in any one experiment by brooding both lots together in the same brooder. To obtain samples of each breed affected as little as possible by differences between individuals and strains, the chicks used in each test were picked at random. Their dams were selected entirely at random. The population from which these chicks came included three strains of Rhode Island Reds and three of White Leghorns. The fowls used to produce chicks for any one test were not the same as those used for the other experiments. Since all the adult breeding stock received the same diet, which was made as complete as possible according to present knowledge of requirements for reproduction in the fowl, any possibility of differential viability in the chicks resulting from differences in the "carry-over" of essential nutrients in the egg was reduced to a minimum.

A diet lacking vitamin B_1 was obtained by the method of Keenan, Kline, Elvehjem, and Hart (*6*) who showed that by autoclaving a mixture of ground grains the vitamin B_1 could be completely destroyed without much loss of vitamin B_2 (riboflavin), or of B_4. The diet used was as follows:

	Pounds per 100
Ground yellow corn	40
Standard wheat middlings	20
Ground buckwheat	15
Wheat-germ meal	2
Soybean oil meal	5
Fish meal	7
Meat scrap	2
Liver meal	3
Alfalfa-leaf meal	3
Ground limestone	2
Salt (NaCl)	.5
High potency cod-liver oil	.5

This diet was used with uniformly good results for all chicks in the regular hatches of the department in 1936. It was autoclaved at 120° C. and 15 pounds pressure for 6 hours. An additional 0.25 percent of high potency cod-liver oil was added after autoclaving. This oil contained 250 units of vitamin D and 3,000 units of vitamin A per gram. The records for the controls proved that the diet not thus treated was entirely satisfactory.

That the data reported are an accurate measure of the requirements for vitamin B_1 and are not complicated by error due to the destruction of other nutrients was shown in a separate experiment with Rhode Island Red chicks. Twenty-five chicks receiving the autoclaved ration died at the mean age of 11 days, and all were dead 14 days after hatching. Of 23 chicks receiving the same ration plus 120 micrograms of synthetic vitamin B_1 (Betabion, Merck) per 100 g of feed, none had died. Of 25 chicks receiving 240 micrograms of synthetic vitamin B_1 per 100 g of feed 2 died at 4 and 5 days of age. The remainder of the chicks appeared quite healthy at the end of the experiment.

The four experiments were so planned that in each one the dietary deficiency was initiated at a different age and (except in experiment 1) in chicks which, before introduction of the deficient diet or afterwards (experiment 2), had been for different periods upon the normal (unautoclaved) diet, with consequent different storages of vitamin B_1 as temporary defence against the deficiency. Only strong, vigorous chicks were used at the start of each trial. The four tests of the two breeds were as follows:

Experiment 1, initiated February 8, 1936. Deficient diet from hatching.

Experiment 2, initiated January 25, 1936. Deficient diet first 2 days; normal feed on the third day, deficient diet thereafter.

Experiment 3, initiated February 1, 1936. Normal feed to 14 days of age; deficient diet thereafter.

Experiment 4, initiated June 10, 1936. Normal feed to 21 days of age; deficient diet thereafter.

In experiments 3 and 4 all the chicks that died and those alive at the end of experiments were sexed. Since no evidence was found that either sex is more susceptible than the other, data for the two sexes were combined for all analyses.

RESULTS

The findings in the first three experiments (table 1) show conclusively that the White Leghorns are more resistant to a deficiency of vitamin B_1 than are the Rhode Island Reds.

TABLE 1.—*Comparative survival of Single Comb White Leghorn and Rhode Island Red chicks on diets lacking vitamin B_1*

Diet and breed	Chicks	Average body weight at—		Age at death	
		1 day	1 week	Range	Mean
Experiment 1. B_1-free diet from hatching	*Number*	*Grams*	*Grams*	*Days*	*Days*
White Leghorn	21	35.0	41.1	5–26	12.86
Rhode Island Red	20	36.1	39.0	9–16	10.85
Experiment 2. B_1-free diet from hatching, normal diet on third day only					
White Leghorn	15	32.0	46.5	15–62	22.13
Rhode Island Red	15	32.3	40.3	13–21	16.93
Experiment 3. Normal diet to 2 weeks, B_1-free diet thereafter:					
White Leghorn	12	36.1	44.1	29–49	34.17
Rhode Island Red	13	35.1	40.8	26–34	29.0

As was to be expected, the mean age at death is lowest for the chicks which never received any normal feed, highest for those on normal feed to 2 weeks of age, and intermediate for those which got normal feed on 1 day only. In each experiment, however, the mean age at death was higher for the White Leghorns than for the Rhode Island Reds. It is noteworthy also that the variation in susceptibility among individuals, as measured by the range in age at death, was much greater in the former than in the latter. In experiments 1, 2, and 3, chicks of the latter breed all died within 8, 9, and 9 days, respectively, whereas deaths in the White Leghorns in the same trials were spread over 22, 48, and 21 days. The most resistant White Leghorn outlived the last surviving Rhode Island Red by 10, 41, and 15 days in experiments 1, 2, and 3, respectively. An exceptional White Leghorn chick in experiment 2 survived to 62 days of age.

It is commonly believed that animals growing most rapidly are more susceptible to nutritional deficiencies than are those in which growth proceeds more slowly. Weights of these chicks at 1 day (table 1) show the samples of the two breeds to be almost identical in size at the beginning of the three tests. The increases in weights of these chicks from hatching to 1 week of age indicate that in all three experiments the White Leghorns grew somewhat more rapidly than the Rhode Island Reds. Ordinarily White Leghorns do not grow more rapidly than Rhode Island Reds in the first week. The differences observed may have resulted from chance or from adverse effects of the vitamin deficiency on the Rhode Island Reds. In any case, the greater resistance of the White Leghorns to a deficiency of vitamin B_1 cannot be ascribed to relatively slow growth in that breed.

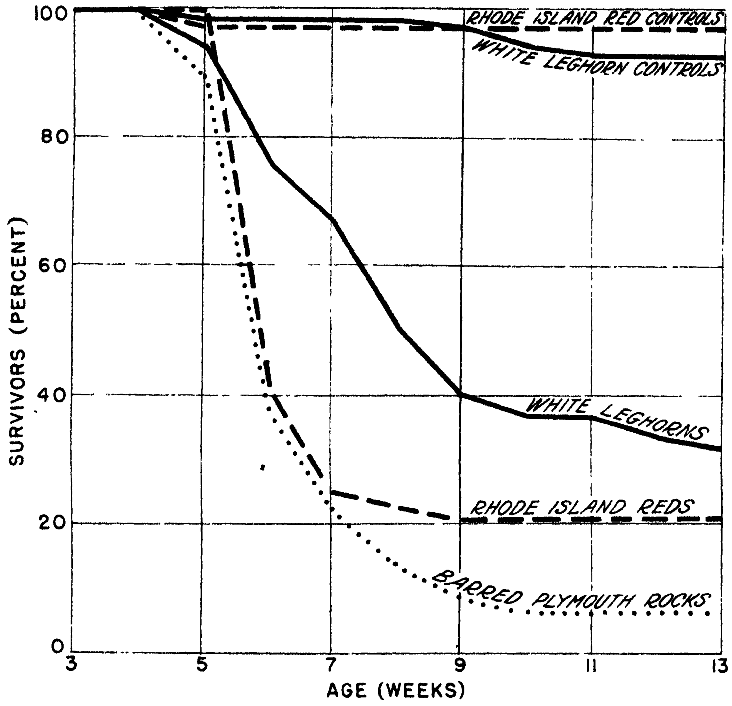

FIGURE 1.—Survival data for 59 White Leghorns, 39 Rhode Island Reds, and 38 Barred Plymouth Rocks placed on vitamin B_1-deficient feed at 3 weeks of age The controls include 128 White Leghorns and 67 Rhode Island Reds.

Similar results were obtained in experiment 4. Since all the chicks did not die, the results in this test are not strictly comparable with those in the first three and are therefore presented separately (figs. 1 and 2). Birds in this experiment were kept in battery brooders till 6 weeks old and confined thereafter in a 10-by-12-foot colony house with access to a wire-floored sun porch. Several cases of cannibalism occurred at from 8 to 12 weeks of age, and it is probable that some of the birds still alive after 10 weeks on the vitamin B_1-free diet (at 13 weeks of age, when the experiment was discontinued) were able to survive because of having received an extra supply of the vitamin from the birds eaten. Since these birds went out of the battery brooders and into the colony house at 6 weeks of age, it is possible that after that time some of the birds which survived the longest acquired a supply of the vitamin by coprophagy. It has not yet been proven, however, that in the fowl, as in the rat (*3*) vitamin B^1 is synthesized in the digestive tract and voided with the feces. However, the critical period in this experiment was when the birds were from 5 to 7 weeks of age, at which time the greatest mortality was experienced by all three breeds. Since cannibalism was not serious till the ninth week, it is improbable that error from this source could have affected in any way the breed difference so conclusively demonstrated at from 5 to 8 weeks (fig. 1).

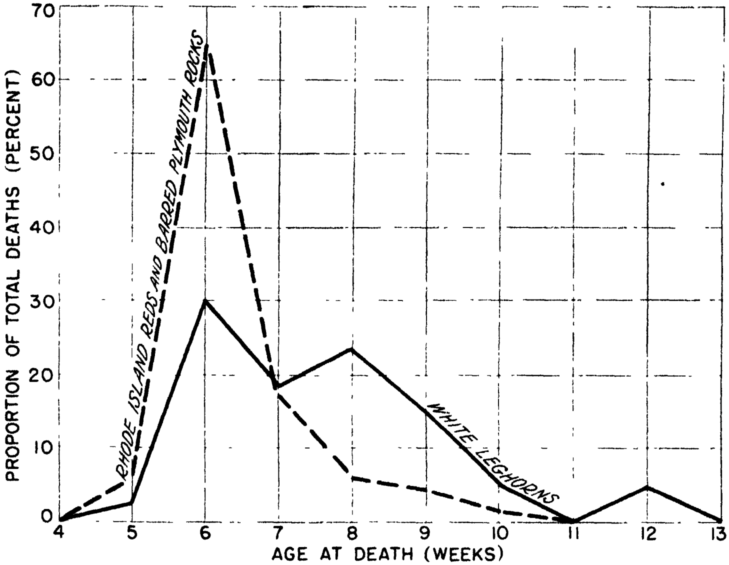

FIGURE 2. Distribution of ages at death in 39 White Leghorns and 65 Rhode Island Reds and Barred Plymouth Rocks that died from deficiency of vitamin B_1.

It is quite evident that whatever storage of vitamin B_1 had been accumulated by these chicks during 3 weeks on normal feed it was quickly exhausted (fig. 1). Some mortality occurred in the fourth week, but in the sixth week (third week on the deficient diet) mortality was extremely high in both the Barred Plymouth Rocks and the Rhode Island Reds. Only 39.5 and 41 percent, respectively, of these survived at the end of the sixth week of the 37 Rhode Island Reds and 34 Barred Plymouth Rocks placed on the deficient diet at 3 weeks of age. In contrast to these, 76.3 percent of the 59 White Leghorns put on the B_1-free diet at 3 weeks were still alive at the end of the sixth week. The percentages of survivors in the Rhode Island Reds, Barred Plymouth Rocks, and White Leghorns at 8 weeks of age were 23.1, 15.8, and 50.8, and at 13 weeks 20.5, 7.9, and 32.2, respectively, of the original numbers in each breed.

The greater resistance of the White Leghorns to a deficiency of this vitamin is demonstrated not only by the lower mortality in that breed but also by the fact that even those that did succumb were able to survive for a longer period than the Rhode Island Reds which died. Analyses of the ages at death up to 13 weeks show that, whereas 64.6 percent of the Plymouth Rocks and Rhode Island Reds that died did so during the sixth week, only 30.8 percent of the White Leghorns that succumbed did so during the sixth week and the rest lived for periods varying up to 7 weeks after the first peak of mortality. As a result the distributions of the ages at death in the two breeds differ markedly (fig. 2). Both curves are skewed; but the curve for the Plymouth Rocks and Rhode Island Reds is leptokurtic, while that for the White Leghorns is platykurtic.

Most of the chicks that died exhibited symptons of vitamin B_1 deficiency including unsteady gait, other signs of neuromuscular incoordination, and, in extreme cases, retraction of the head in characteristic opisthotonos. The birds appeared not to relish the autoclaved feed. This may have resulted from the fact that autoclaving made the mash dark in appearance and (presumably) less palatable, particularly to those chicks which had received normal feed before going on the autoclaved diet. On the other hand, the condition of anorexia may have resulted, in part or entirely, directly from lack of vitamin B_1 as Cowgill (*2*) has shown may happen in other species.

Other evidence of a deficiency of vitamin B_1 was provided by the retardation in growth of the experimental chicks. At 5 weeks of age, just prior to the onset of heavy mortality, the mean weights of the White Leghorns and Rhode Island Reds were respectively 87 and 97 g less than the normal weights for these breeds at 5 weeks, as determined by Card and Kirkpatrick (*1*). Since at 3 weeks of age, when these chicks were placed on the deficient diet, they had exceeded Card and Kirkpatrick's normal weights by 16.5 g in the White Leghorns and 13.7 g in the Rhode Island Reds, the experimental chicks obviously showed at 5 weeks a marked retardation of growth such as is commonly associated with a deficiency of vitamin B_1.

It seems reasonable to conclude, therefore, that the deaths in these chicks resulted primarily from polyneuritis attributable to the deficiency of vitamin B_1.

BREED DIFFERENCES ON A NORMAL DIET

It seemed possible that the differences between breeds with respect to mortality rate might be present even in birds raised under optimum conditions. If this were so, the differences found in experiments 1 to 4 might reflect a general debility of the Rhode Island Reds in comparison with White Leghorns, rather than a special susceptibility to a deficiency of vitamin B_1. To answer this question a comparison was made of control populations of each breed drawn at random from the several thousand chicks hatched for other purposes from the same strains as those providing the chicks for these experiments.

The mortality curves for these populations show that the strains of White Leghorns and Rhode Island Reds used did not differ in this respect when on normal feed (fig. 1). Up to 3 weeks of age none of either breed died, and at 13 weeks the deaths were 1.5 percent in the Rhode Island Reds and 4.8 percent in the White Leghorns. The diet used for these controls was the same as that of the experimental birds except that it was not autoclaved. It was obviously satisfactory.

It seems safe to conclude that chicks of the two breeds used did not differ in mortality from other causes and that the differences observed in them on the experimental diets can be attributed solely to a difference between the breeds in susceptibility to a deficiency of vitamin B_1.

INDEPENDENCE OF SUSCEPTIBILITY AND BODY SIZE

The demonstration by Nichita et al. (*9, 10*) that adult Rhode Island Reds are more susceptible than adult White Leghorns to a lack of vitamin B_1 indicates a difference between breeds but raises the question whether or not that difference depends upon anything other than the difference in size of those two breeds. Cowgill (*2*) has presented some data indicating that in the larger species the amount of vitamin B_1 required per animal per day is somewhat less in proportion to body weight than in small ones. If this same principle were applicable to intraspecific races differing markedly in size, one would expect a somewhat lower requirement per unit of weight for Rhode Island Reds than for White Leghorns since the former breed exceeds the latter in weight at maturity by an average amount (in females) of around 900 g. This would be the reverse of what has actually been found.

However, the validity of Cowgill's formulas, which are based upon rather hypothetical "maximum normal weights" of the species considered, is open to question. Direct proof that the lower requirement of vitamin B_1 by White Leghorns is independent of their smaller body size at maturity is provided when the experimental animals are of the same size in the breeds compared. It was with this fact in mind that the experiments reported in this paper were carried out with young chicks. Comparisons of growth in White Leghorns and Rhode Island Reds made by Card and Kirkpatrick (*1*) and by Kempster and Parker (*7*) have shown that chicks of the two breeds do not differ in size till after 4 weeks. The present writers' data agree with their findings.

It follows, therefore, that in experiments 1 and 2, where all chicks but one died earlier than 4 weeks of age, and in experiment 3, where the mean age at death in the resistant white Leghorns was not quite 5 weeks, the markedly superior ability of the White Leghorns to withstand a deficiency of vitamin B_1 is a distinct racial characteristic entirely independent of body size. As it happened, the White Leghorns were slightly heavier than the Rhode Island Reds at 1 week of age in these three tests (table 1). In experiment 4, where the deficient diet was not introduced till the chicks were 3 weeks of age, the chicks of the two breeds on hand at 5 weeks of age weighed practically the same, 142.9 g for the Leghorns and 136.4 g for the Rhode Island

Reds. It was in the succeeding 2 weeks that both breeds experienced their heaviest losses (figs. 1 and 2). Obviously the superiority of the White Leghorns in this test was in no way related to body size.

CROSS OF RESISTANT × SUSCEPTIBLE BREEDS

While the primary object of this investigation was to determine whether or not there is a difference between White Leghorns and Rhode Island Reds with respect to their requirement of vitamin B_1, a test was also made to get some idea of the genetic basis for the lower requirement of the White Leghorns. The chicks used were from a cross of Rhode Island Red ♀♀ × White Leghorn ♂. These were tested concurrently with the purebreds in experiment 4 and in exactly the same manner. Mortality in the 53 cross-bred chicks was 73 percent up to 13 weeks of age, when the test was discontinued. The mortality in the same period was 68 percent for purebred White Leghorns and 79.5 percent for purebred Rhode Island Reds. It would appear, therefore, that the crossbred chicks were intermediate between the parent breeds in requirement of vitamin B_1. While further data are necessary to prove the point, this is some indication that the comparatively low requirement of this vitamin by the White Leghorns depend upon multiple factors.

DISCUSSION

These experiments with chicks yielded the same results with respect to breed differences in requirement of vitamin B_1 as did those of Nichita and Iftimesco (*9*) and Nichita et al (*10*) with adult birds. Since the strains of White Leghorns and of Rhode Island Reds used by the Rumanian workers were quite different from those used by the present authors, and since the birds of each breed used by the present writers came from more than one strain, there can be no doubt that the difference observed is really one between breeds and not merely between strains. The low requirement of vitamin B_1 is as truly a genetic breed character as are the dominant white of White Leghorns and the restricted black of the Rhode Island Reds. Whether it is peculiar to White Leghorns or occurs in all Leghorn varieties regardless of color remains to be determined.

The physiological basis for this special attribute of the White Leghorns is as yet quite unknown, and little or no explanation of its persistence as a breed characteristic can be offered. It is obviously different from practically all other distinguishing breed characteristics in that the latter are maintained by artificial selection, whereas it is impossible to see how the poultrymen, either modern or primitive, who helped to establish the White Leghorn breed, could have consciously selected for a lower requirement of vitamin B_1 as they did for morphological characteristics.

The character under consideration is not the only physiological one in which White Leghorns and Rhode Island Reds differ. Hutt (*4*) has shown that these two breeds differ in the rates at which the body temperature rises during the first 9 days after hatching. He (*5*) has also shown that adult White Leghorns are much more resistant to extreme heat than are Rhode Island Reds and Barred Plymouth Rocks. In a discussion of these and other physiological differences between breeds, the junior author (*5*) has suggested ways in which such differences might have become established, but none of these can yet be considered as anything more than conjecture. The differences are probably between all Leghorns as one class and the so-called heavy breeds as another, rather than merely between the White Leghorns and the Rhode Island Reds.

So far as the writers can ascertain, apart from the work of Nichita and his coworkers, confirmed in this paper, it has not previously been demonstrated that breeds of the domestic fowl vary in their requirement of certain vitamins. Since most recent investigations of the nutritional requirements of fowls are based upon experiments with White Leghorns, there is much doubt about the extent to which findings reported for that breed are applicable to Rhode Island Reds and to other heavy breeds. There has been a tendency in recent years to establish standard requirements of various vitamins for poultry, i. e., so many units of vitamin A, of vitamin D, or of riboflavin per gram of feed. It is obvious that, with respect to vitamin B_1, any such requirement determined from experiments with White Leghorns only would not be accurate for Rhode Island Reds and vice versa.

Assay of vitamin B_1 with pigeons, according to the method of Kinnersley, Peters, and Reader (*8*) is now a standard procedure. These investigators tested pigeons of several different colors, but, so far as the present writers are aware, no experiments have yet been made to determine whether all of the many different breeds of pigeons have the same requirement of this vitamin, or whether some would yield entirely different results in such an assay. It is not impossible that some of the differences in such assays reported from different laboratories have arisen because the pigeon is not yet so well standardized as the commonly used rats of the Wistar Institute strain.

SUMMARY

In each of four experiments the ability of White Leghorn chicks to survive on a diet deficient, or lacking, in vitamin B_1 was greater than that of Rhode Island Reds. When chicks received the deficient diet from hatching, the mean ages at death were 12.9 days for the White Leghorns and 10.8 days for the Rhode Island Reds. For chicks similarly treated but given nondeficient feed on the third day only, the corresponding mean ages were 22.1 and 16.9 days. When deficient feed was supplied after the chicks had been for 2 weeks on the normal diet, the mean ages at death were 34.1 days in the White Leghorns and 29 days in the Rhode Island Reds. Typical symptoms of polyneuritis were observed.

Among chicks on normal feed to 3 weeks of age and the deficient diet thereafter, the percentages surviving to 13 weeks of age were 32 for the White Leghorns, 20.5 for the Rhode Island Reds, and 8 for Barred Plymouth Rocks.

In the White Leghorns deaths were delayed and spread out over a longer period, while in the heavy breeds they occurred earlier and were clustered somewhat narrowly around the mean age at death.

The superior resistance of the White Leghorns to this dietary deficiency is shown to be independent of body size and is not caused by differential mortality from causes other than polyneuritis.

F_1 progeny from a cross of Rhode Island Red ♀♀ × White Leghorn ♂ were intermediate between the parent breeds with respect to resistance to a deficiency of this vitamin.

These findings, along with those of Nichita and his coworkers in Rumania, prove that White Leghorns differ from Rhode Island Reds and Barred Plymouth Rocks in possessing as a hereditary breed characteristic a marked resistance to a deficiency of vitamin B_1.

The significance of this fact in poultry feeding, in experimental work in nutrition, and in the use of birds for quantitative assays of vitamin B_1 is discussed.

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THE USE OF THE ANALYSIS OF COVARIANCE AND ITS LIMITATION IN THE ADJUSTMENT OF YIELDS BASED UPON STAND IRREGULARITIES[1]

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INTRODUCTION

In the course of investigations dealing with the breeding of vegetable crops, where progenies were tested in comparision with commercial strains, more perfect stands were very frequently obtained from seed of the latter. This was particularly true for hand-pollinated hybrid sweet corns. Even though replanting can be used, it is often difficult to obtain perfect stands with such widely spaced transplanted crops as tomatoes and muskmelons. Methods were investigated, therefore, for correcting for stand irregularities on plots which had been so designed that statistical interpretation could be made by the analysis of variance.

EFFICIENCY GAINED BY ADJUSTING YIELDS

The influence of stand on yield has been investigated by numerous workers. Bryan (*4*)[2] working with corn has shown that stand influenced yield more significantly than border effect, and he states that "adjustment for perfect hill stands reduced the number of hills required for any degree of precisions by 18.9 percent." Matthews (*7*) and Gillis (*6*) have shown that wider spacings have reduced yields of snap beans and lima beans. Beattie and Boswell (*2*) found that machine seeding of row crops did not insure perfect stands, and they found a high correlation between stand and yield of both carrots and onions grown on muck soil. The influence of stand on yield has not been thoroughly investigated for all vegetable crops, and this correlation must be established before any degree of precision will be attained in correcting yield on the basis of stand. Beattie, Boswell, and Batten (*3*) have shown the absence of correlation between stand and yield of peanuts.

The methods of adjusting yield used in this paper are not original and have been presented by various statistical workers, but it is hoped that presentation of examples of the use of the regression coefficient obtained from variance and covariance as a means of adjusting yields for irregularities of stand will be of value to other workers with vegetable crops for more adequately interpreting the results of their field experiments.

The data from randomized plots of 26 hybrid strains and 1 open-pollinated strain of yellow sweet corn will be used to illustrate one case where the efficiency of the experiment has been increased by adjustment of yields on the basis of stand. The yield-record sheets from 5 of the 108 plots were lost, and the method of estimation for missing plots, as suggested by Yates (*11*) and Allan and Wishart (*1*), has been used to determine estimates of these plots. This method is (*Number of treatments* × *total of treatments*) + (*Number of replications* × *total of replications*) − *total of all plots* divided by (number treated − 1) × (number replicated − 1)[3]

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[2] Italic numbers in parentheses refer to Literature Cited, p. 328.

The data on yields and number of plants for the 108 plots are presented in table 1.

TABLE 1.—*Yield of marketable ears and numbers of plants (stand) of 108 plots with mean yields and stands for 27 strains of yellow sweet corn grown in 1937 (unadjusted)*

Item	Yield and stand for strain No.—													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Replication 1:														
Yield (*Y*)	56	31	21	23	50	60	46	58	31	42	31	61	47	56
Stand (*X*)	77	68	61	83	70	84	72	81	79	71	61	73	58	81
Replication 2														
Yield (*Y*)	64	29	32	20	59	50	50	50	39	53	36	55	65	65
Stand (*X*)	80	76	72	88	89	92	82	83	74	89	68	68	64	90
Replication 3.														
Yield (*Y*)	56	30	24	18	60	47	55	62	48	60	34	62	62	58
Stand (*X*)	74	83	82	78	78	78	84	96	75	89	81	85	71	102
Replication 4														
Yield (*Y*)	36	32	[1] 24	[1] 19	39	[1] 50	55	55	40	49	34	48	59	62
Stand (*X*)	57	61	73	78	52	88	97	77	71	58	67	77	67	97
Strain yield totals	212	122	101	80	208	207	206	225	158	204	135	226	233	241
Strain stand totals	288	288	288	327	289	342	335	337	299	307	277	303	260	370
Mean yield	53.0	30.5	25.3	20.0	52.0	51.8	51.5	56.3	39.5	51.0	33.8	56.5	58.3	60.3
Mean stand	72.0	72.0	72.0	81.8	72.3	85.5	83.8	84.3	74.8	76.8	69.3	75.8	65.0	92.5

Item	Yield and stand for strain No.—													Replication totals	
	15	16	17	18	19	20	21	22	23	24	25	26	43	Yield	Stand
Replication 1															
Yield (*Y*)	52	54	46	24	55	57	46	55	39	67	64	63	45	1,280	----
Stand (*X*)	94	72	63	34	80	73	78	75	65	74	96	81	64	----	1,968
Replication 2.															
Field (*Y*)	73	50	55	32	57	61	55	51	48	79	57	72	50	1,407	----
Stand (*X*)	98	82	62	62	81	73	84	64	79	88	80	98	65	----	2,131
Replication 3															
Yield (*Y*)	74	65	[1] 56	50	50	37	65	52	50	66	66	74	56	1,437	----
Stand (*X*)	110	97	69	60	94	81	80	84	77	90	92	103	78	----	2,271
Replication 4															
Yield (*Y*)	64	64	51	26	[1] 52	41	62	54	51	53	60	73	53	1,306	----
Stand (*X*)	92	82	69	24	77	68	73	76	66	85	87	85	71	----	1,975
Strain yield totals	263	233	208	132	214	196	228	212	188	265	247	282	204	5,430	----
Strain stand totals	394	333	263	180	332	295	315	299	287	337	355	367	278	----	8,345
Mean yield	65.8	58.3	52.0	33.0	53.5	49.0	57.0	53.0	47.0	66.3	61.8	70.5	51.0	50.1	----
Mean stand	98.5	83.3	65.8	45.0	83.0	73.8	78.8	74.8	71.8	84.3	88.8	91.8	69.5	----	77.3

[1] Estimation of missing data.

The analysis of variance and covariance for the yields of marketable ears and for stand (number of plants per plot) is given in tables 2 and 3.

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[3] This formula was used once for estimating each missing plot yield.

TABLE 2 —*Analysis of variance and covariance for 1937 hybrid sweet-corn trials for unadjusted data in table 1*

Source	Degree of freedom	Yield, Y		Stand, X		Covariance, XY	
		Sum of squares	Variance	Sum of squares	Variance	Sum of products	r=correlation coefficient
Total	[1]102 107	20,174		18,475		9 766	
Replications	3	646	215.33	2,315	[2]771 7	1,178	[2]0.963
Strains	26	16,206	[2]623 31	11,752	[2]452 0	7,326	[2] 531
Error	[1]73 78	3,322	45.51	4,408	56 5	1,262	[3].330
		Strain F=13.70 [4]		Strain F=8 00		b (regression coefficient)=0 2863, obtained from (xy) and $(x)^2$ for error	
		[3]1 67 [2]2 07		[3]1.67 [2]2.07			
Standard error of experiment		6.75		7 52			

[1] Degrees of freedom for yield, because of estimating 5 missing yields is 102. Degrees of freedom for stand is 107

[2] 1-percent point. [3] 5-percent point.

[4] See table 3 for "best" value of F for testing significance between strain means.

TABLE 3.—*Analysis for testing significance between strain-yield means because of missing plots* [1]

Source	Degrees of freedom	SS (sum of squares)	Variance
Totals [2]	102	18,444.41	45.51
Error [3]	73	3,322 00	
Block+treatments	29	15,122 41	
Blocks only [4]	3	498 83	
Difference	26	14,623 58	562 45

$$F=\frac{562\ 45}{45.51}=12.4.$$

[1] See Yates (*11*).

[2] Obtained from original yields not including estimates of missing yields.

[3] From table 2.

[4] Obtained from block totals without the estimates of missing yields.

It is evident that the correlation coefficients between stand and yield in table 2 are significant. This was to be expected as seed is often a limiting factor when planting hand-pollinated single crosses and invariably more seed is planted and a better stand obtained with established commercial hybrids. That there is a definite correlation between stand and yield is further borne out by the highly significant correlation coefficient of the error components shown in the last column of table 2. Since this is true, it would follow that adjustment of yields on the basis of stand might logically change the mean yields and ranks of some of the hybrids. If the regression coefficient is computed, it is possible by the use of the following equation, suggested by Miles and Bryan (*8*), to adjust the yields of marketable ears for each of the 108 plots:

$Y'=Y-b\ (X-Mx)$ where
Y'=adjusted yield
Y=actual individual yields
X=actual individual stands
Mx=mean of all stands
b=regression coefficient, obtained from $\Sigma(xy)$ and $\Sigma(x)^2$ for error.

The adjusted yields, carried out to two decimal places derived by the foregoing equation, are given in table 4

TABLE 4.—*Yield of marketable ears of 108 plots of 27 strains of sweet corn adjusted on the basis of stands in table 1*

Item	Yield of Strain No —													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Replication 1	56.09	33.66	25.66	21.37	52.09	58.08	47.52	56.94	30.51	43.80	35.66	62.23	52.52	54.94
2	63.23	29.37	33.52	16.94	55.65	45.80	48.66	48.37	39.94	49.65	38.66	57.66	68.80	61.37
3	56.94	28.37	22.66	17.80	59.80	46.80	53.08	56.65	48.66	56.65	32.94	59.80	63.80	50.94
4	41.81	36.66	25.23	18.80	46.24	46.94	49.37	55.09	41.80	54.52	36.95	48.09	61.95	56.37
Strain means	54.52	32.02	26.77	18.73	53.45	49.41	49.66	54.26	40.23	51.16	36.05	56.95	61.77	55.91

Item	Yield of Strain No —													Replication	
	15	16	17	18	19	20	21	22	23	24	25	26	43	Total	Sums of squares
Replication 1	47.22	55.52	50.09	36.38	54.23	58.23	45.80	55.66	42.52	67.94	58.65	61.94	48.80	1,314.05	67,505.06
2	67.08	48.66	59.38	36.38	55.94	62.23	53.08	54.80	47.51	75.94	56.23	66.08	53.52	1,394.45	76,508.76
3	64.65	59.37	58.37	54.95	45.22	35.94	64.23	50.08	50.09	62.37	61.80	66.65	55.80	1,384.41	75,448.89
4	59.80	62.66	53.37	41.24	52.09	43.66	63.23	54.37	54.23	50.80	57.23	70.80	54.80	1,338.10	69,746.42
Strain means	59.69	56.55	55.30	42.24	51.87	50.01	56.59	53.73	48.59	64.26	58.48	66.37	53.23	5,431.01 [1] 50.29	289,209.13

[1] General mean.

After the adjusted individual plot yields were determined an analysis of variance for adjusted yields was then carried out; this is shown in table 5, and an analysis for securing a test of significance between adjusted strain yield means is presented in table 6.

TABLE 5.—*Analysis of variance of yields of marketable ears for 1937 hybrid sweet corns (individual plot yields adjusted)*

Source	Degrees of freedom	Sum of squares	Variance [1]	Standard error
Total	[1] 101	16,098		
Replication	3	161	53.7	
Strain	26	12,977	[2] 499.1	
Error	72	2,960	41.1	
			F=12.1	
			[3] 1.67	
			[4] 2.07	
				6.41

[1] 1 degree of freedom less than given in table 2 as 1 degree was lost in the use of the regression coefficient *b*.
[2] Significant to 1-percent level
[3] 5-percent point
[4] 1-percent point.

TABLE 6.—*Analysis for securing a test of significance between adjusted strain yields*

Source of variation	Degrees of freedom	Sums of squares and products			Errors of estimate for yields		
		Sx^2	$S(xy)$	Sy^2	Sum of squares	Degrees of freedom	Variance
Strain [1]	26	11,752	7,326	16,206			
Error [1]	73	4,408	1,262	3,322	2,960.69	[2] 72	[3] 41.1
Strain plus error	99	16,160	8,588	19,528	[3] 14,964.03	98	
Strain					[4] 12,003.34	26	461.7

$$F=\frac{461.7}{41.1}=11.2$$

[1] These values came from table 2.
[2] Because 1 degree of freedom is used up by using *b*
[3] Found by employing the formula $Sy^2-\frac{S^2(xy)}{Sx^2}$=sum of squares of errors of estimate. The quantity Sy^2 is the sum of squares of *y* for strain + error, $S^2(xy)$ is the square of the sum of products for strain + error, and Sx^2 is the sum of squares of *x* for strain + error This can also be found by using the formula $S(y^2)-2bS(xy)+b^2S(x^2)$, where *b* is found from the line strain + error, and the sums of squares and sum of products are taken from the strain + error line in the above table
[4] Found by the subtraction [14,964.03−2,960.69=12,003.34].

It can be seen readily by comparing tables 2 and 5 that the variances have been reduced throughout, but the effectiveness of the method is evident in the reduction of the standard error of the experiment. Before adjustment 4 hybrids were significantly higher, 5 significantly lower, and 17 showed no significant differences, with reference to open-pollinated Golden Bantam. After the yield had been adjusted on stand, 2 hybrids were higher, 6 lower, and 18 not significantly higher or lower than open-pollinated Golden Bantam.[4]

As a result of adjustment two hybrids which previously had appeared significantly higher than the check, open-pollinated Golden Bantam, were found to be not significant. There was no change in the rank of the two highest producing varieties. The ranks of the first seven high-producing strains before and after adjustment are presented in table 7.

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[4] The standard error of the difference between two adjusted yield means y_p and y_q is

$$s\sqrt{\frac{2}{n}+(\bar{x}_p-\bar{x}_q)^2/\Sigma(x-\bar{x})^2},$$

where s is the square root of the variance for error, $\Sigma(x-\bar{x})^2$ is sum of squares for stand in same line with error, n the number of replications, $\bar{x}_p$ the mean of stand for p^{th} variety, $\bar{x}_q$ the mean of stand for the q^{th} variety This method was suggested by Goulden.[5]

[5] GOULDEN, C. H. METHODS OF STATISTICAL ANALYSIS. Rev. ed., 209 pp. Minneapolis. 1937. [Mimeographed.]

TABLE 7.—*Ranking before and after adjusting for stand for the seven highest producing strains*

Variety key No.	Ears per plot before adjustment	Rank	Ears per plot after adjustment	New rank	Variety key No	Ears per plot before adjustment	Rank	Ears per plot after adjustment	New rank
	Number		*Number*			*Number*		*Number*	
26	70.5	1	66.4	1	25	61.8	4	58.5	5
24	66.3	2	64.3	2	16	58.3	[1] 5	56.6	[1] 6
13	58.3	[1] 5	61.8	3	21	57.0	7	56.6	[1] 6
15	65.8	3	59.7	4					

[1] Tied.

It might be of interest from the canners' standpoint to point out that 13 hybrids produced a significantly higher yield of green unhusked corn per acre, and only 8 which were significantly lower than open-pollinated Golden Bantam. Likewise 13 hybrids were significantly higher and only 3 significantly lower than Golden Bantam for yield of husked green corn per acre.

The above example illustrating methods for correcting the yields of individual plots and setting up the analysis of variance pertaining to adjusted yields has been presented as a background for a much simpler and much less laborious method of obtaining the analysis of variance concerning adjusted data from the statistics presented in table 2. Using Sanders' (*9*) formula for finding sum of squares for adjusted yields, $b^2S(x)^2-2bS(xy)+S(y^2)$,[6]
Where $S(x)^2$ is the sum of squares for stand and $S(y)^2$ is the sum of squares for yield and $S(xy)$ is the sum of products, and substituting the statistics given in table 2 we get the following:

$(0.2863)^2\times 18{,}475-[2(0.2863)\times 9{,}766]+20{,}174=16{,}096.38$ for the new total sum of squares. This same formula is used to work out the new sum of squares for replications, strains, and error. The analysis of variance for adjusted yields worked out by this method is given in table 8.

TABLE 8.—*Analysis of variance for 1937 hybrid sweet corns adjusted for stand by short method* [1]

Source	Degrees of freedom	Sum of squares	Variance [1]	Source	Degrees of freedom	Sum of squares	Variance [1]
Total	101	16,096.38		Strain	26	12,974.44	[2] 499.0
Replication	3	161.22	53.7	Error	72	2,960.72	41.1

[1] See table 6 for test of significance between adjusted means. Standard error=6.41.
[2] Significant to 1-percent level.

A comparison of tables 5 and 8 shows that the two analyses of variance are practically identical. The two total sums of squares differ by less than 2 units and sums of squares for replication are nearly identical. The sums of squares for strain and error differ by less than 3 units. It is necessary, in the calculation of plot yields by the first method, to work out the yields to three decimal places and to use four decimal places in the regression coefficient in order to make the two methods check. The adjusted means of the strains can then be obtained by use of the formula already given $Y'=Y-b\ (X-Mx)$.[7]

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[6] b is $S(xy)$ for error divided by $S(x)^2$ for error and is used to find the sum of squares in table 8.
[7] In this formula Y' now designates adjusted yield mean, Y the observed yield mean, and X the stand mean.

This short method originally given by Sanders (*9*) has later been described by Fisher (*5*), Snedecor (*10*), and Miles and Bryan (*8*).

SIGNIFICANCE BETWEEN STAND MEANS—NOTHING GAINED BY ADJUSTING

The advisability of adjusting seeded vegetable-plot yields on the basis of stand must be determined by the investigator. This can often be done by observation. If there is a fairly high variation due to soil heterogeneity and there are no appreciable differences in stand, usually nothing will be gained by adjustment. The analysis of variance for nine hydrids and one open-pollinated yellow sweet corn planted in 1935 and arranged in a 10 by 10 Latin square (table 9) showed a nonsignificant yield variance for strains, yet the strain variance for stand was significant. Would adjustment of yield on stand in this case disclose any significant strain means? The correlation coefficient *r* pertaining to error was found to be 0.247 (table 10), and with 71 degrees of freedom slightly exceeds Fisher's 5-percent point (*5*). The regression coefficient ($b=0.442$) was determined, and the new analysis of variance was made. This is presented in table 10 in comparison with the analysis of the original data.

TABLE 9.—*1935 sweet corn data on yield of marketable ears and stand of plants per plot for nine hybrids and one open-pollinated sweet corn planted in a Latin Square*

	1	2	3	4	5	6	7	8	9	10	Total of rows
Strain No.	C-4	C-7	C-3	C-6	C-8	C-10	C-5	C-1	C-9	C-2	
Yield	37	41	41	49	29	38	33	32	40	45	385
Stand	42	43	25	42	39	39	42	43	43	40	398
Strain No.	C-2	C-4	C-7	C-1	C-6	C-5	C-8	C-9	C-10	C-3	
Yield	43	40	48	40	46	31	41	34	30	34	387
Stand	42	47	45	46	39	48	46	45	39	31	428
Strain No.	C-1	C-8	C-9	C-5	C-4	C-2	C-3	C-7	C-6	C-10	
Yield	46	42	42	40	35	36	37	42	41	47	408
Stand	46	47	47	43	48	44	35	46	41	39	436
Strain No.	C-5	C-1	C-10	C-4	C-7	C-8	C-2	C-6	C-3	C-9	
Yield	49	46	40	36	45	45	43	46	38	47	435
Stand	41	44	44	42	45	43	45	48	37	44	433
Strain No.	C-10	C-5	C-4	C-9	C-2	C-3	C-6	C-8	C-7	C-1	
Yield	48	43	40	46	47	41	39	40	38	43	425
Stand	43	46	45	48	48	40	41	44	41	46	442
Strain No.	C-3	C-9	C-6	C-2	C-1	C-7	C-10	C-5	C-4	C-8	
Yield	42	48	40	40	50	51	35	53	42	50	451
Stand	36	41	42	41	45	46	36	46	48	46	427
Strain No.	C-9	C-3	C-1	C-10	C-5	C-6	C-7	C-2	C-8	C-4	
Yield	48	46	47	49	49	46	43	35	45	38	446
Stand	47	41	48	46	45	46	48	45	48	48	462
Strain No.	C-8	C-10	C-5	C-7	C-3	C-1	C-9	C-4	C-2	C-6	
Yield	44	48	44	52	48	48	45	47	42	44	462
Stand	48	46	47	46	48	47	48	47	45	45	467
Strain No.	C-7	C-6	C-2	C-8	C-10	C-9	C-4	C-3	C-1	C-5	
Yield	42	44	45	42	51	46	40	52	45	45	452
Stand	48	47	48	46	46	48	48	45	47	45	468
Strain No.	C-6	C-2	C-8	C-3	C-9	C-4	C-1	C-10	C-5	C-7	
Yield	50	41	47	51	48	44	45	40	39	55	460
Stand	48	45	47	41	48	48	47	47	47	47	465
Total of—Stand	441	447	438	441	451	449	436	456	436	431	4426
Total of—Yield	449	439	434	445	448	426	401	421	400	448	4311
Strain No.	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	[1]C-10	
Total for strains—Stand	459	443	379	463	450	439	455	454	459	425	4426
Total for strains—Yield	442	417	430	399	426	445	457	425	444	426	4311
Mean for strains—Stand	45.9	44.3	37.9	46.3	45.0	43.9	45.5	45.4	45.9	42.5	44.26
Mean for strains—Yield	44.2	41.7	43.0	39.9	42.6	44.5	45.7	42.5	44.4	42.6	43.11
Adjusted yield means	43.5	41.7	45.8	39.0	42.3	44.5	45.2	42.0	43.7	43.4	

[1] Strain C-10 is open-pollinated Golden Bantam.

TABLE 10.—*Analysis of variance and covariance for 1935 sweet corn*

Source of variation	Degrees of freedom	X=stands		XY	Y=yields		Correlation coefficient	Errors of estimate of yield		
		Sum of squares	Variance	Sum of products	Sum of squares	Variance		Sum of squares	Degrees of freedom	Variance
Total	99	1,559.24		699.14	2,813.79			[1] 2,500.31		
Between row means	9	470.04	52.23	472.14	750.09	83.34	[2] 0.795	[1] 275.84		
Between column means	9	55.84	6.20	15.14	315.69	35.08	.114	[1] 311.59		
Between strain means	9	566.04	[3] 62.89	5.34	246.89	27.43	.014	[1] 246.84	9	
Error	72	467.32	6.49	206.52	1,501.12	20.85	[4] .247	[1] 1,409.99	71	19.86
Strain plus error	81	1,033.36		211.86	1,748.01			1,704.57	80	
Strain	9							294.58	9	32.73
F for strain			[3] 9.69			1.32				1.65
b for error				0.442						
Standard error						[2] 4.57				[5] 4.46

[1] These values were found by using the formula where *b* is found from the sums of squares and sums of products. The first 4 values are not needed for finding a test for testing yield means for adjusted yields. These 4 will not be given in the remaining tables.

[2] Used in testing differences between yield means before adjustment.

[3] Significant at 1-percent level.

[4] Significant at 5-percent level

[5] Used in testing differences between adjusted yield means.

The adjustment of yields on stand reduced the variance due to rows, and increased the variance due to strains, yet *F* for strain was not significant. The adjusted strain means, however, failed to show any that were either significantly higher or lower than open-pollinated Golden Bantam (C-10). The difference between the highest and lowest yielding strains was significant and there were three yields significantly larger than the lowest.

This is an example where the value of *F* indicated no significant differences between the means, yet the tests showed significant differences between the lowest yield means and the highest yield means. This was true before and after adjustment for stand. Nothing was gained here in adjusting for stand. The lowest mean yield was significantly different from four yield means before adjusting for stand.

NO SIGNIFICANCE BETWEEN STAND MEANS—NOTHING GAINED BY ADJUSTING

It is possible in randomized plots to obtain an insignificant treatment (fertilizer, strains, etc.) variance and yet obtain significant differences between some of the treatment means. This is likely in fertility plots where a large number of different fertilizers are applied and especially so if the units are doubled for each change in analysis or rate of application. Often there is no significant difference between the general mean and variety or treatment means, while there are significant differences between variety or treatment means. Horticulturally, we are more interested in comparing the treatments with a check (no treatment or grower's treatment) or with one another than with the general mean.

A good example of a case of this kind is the fertilizer analysis and rate of application experiment on tomatoes grown for canning at the Michigan station in 1937. Georgia plants were set in 4 randomized blocks, and 11 different treatments were used along with the check.

The original data on yield and stand are given in table 11, and the analysis of variance and covariance for yield of U. S. No. 1 and No. 2 tomatoes and stand is presented in table 12.

TABLE 11.—*Yield in pounds per plot of U. S. No. 1 and No. 2 grade combined and stand of plants per plot for 1937 tomato fertilizer experiment*

UNDER DIFFERENT TREATMENTS (T)

Item	Yield and stand of treatment—												Replication totals	
	T-1	T-2	T-3	T-4	T-5	T-6	T-7	T-8	T-9	T-10	T-11	T-12	Yield	Stand
Replication 1														
Yield	177	161	254	217	145	214	155	176	133	262	190	113	2,197	
Stand	20	20	19	20	20	20	20	20	20	20	20	19		238
Replication 2														
Yield	99	199	169	189	139	146	148	192	94	124	224	223	1,946	
Stand	20	20	16	20	20	20	20	20	20	19	19	20		234
Replication 3														
Yield	78	133	102	132	242	176	172	213	118	182	181	193	1,922	
Stand	20	18	20	17	20	20	20	20	20	20	20	20		235
Replication 4														
Yield	164	203	195	214	107	210	224	212	116	187	179	149	2,160	
Stand	20	20	20	20	20	18	20	19	20	19	20	20		236
Mean yield	129.5	174.0	180.0	188.0	158.2	186.5	174.8	198.2	115.2	188.7	193.5	169.5	[1] 171.4	
Mean stand	20.0	19.5	18.8	19.2	20.0	19.5	20.0	19.8	20.0	19.5	19.8	19.8		[1] 19.7
Adjusted yield means	127.2	175.5	186.9	191.8	155.9	188.0	172.5	197.4	112.9	190.2	192.4	168.7		

[1] General means

TABLE 12.—*Analysis of variance and covariance for 1937 tomato fertilizer plots in table 11*

Source of variation	Degrees of freedom	X=Stands		XY	Y=Yields		Correlation coefficients	Errors of estimate of yield		
		Sum of squares	Variance	Sum of products	Sum of squares	Variance		Sum of squares	Degrees of freedom	Variance
Total	47	33		41	92,111					
Between replication means	3	1	0.333	53	5,063	1,688	0.745			
Between treatment means	11	6	.545	−211	28,763	2,615	−.508		11	
Error	33	26	.788	199	58,285	1,766	.162	56,761.9	32	1,774
Treatment plus error	44	32	1.333	−12	87,048			87,043.5	43	
Treatment	11							30,281.6	11	2,753
F for treatment			1.45			1.48				1.55
b for error					7.65					
Standard error						42.0				42.1

From an examination of table 12 it is evident that treatment F for stand is not significant and that probably there would be no significant differences between the means of the treatments. Since the check produced the lowest yield with one exception, there were two treatments which produced more than the ±3.64 tons compared to the check necessary for significance, and these are beyond the 5-percent point. Three additional treatments had means which approached the ±3.64-ton difference for significance. Even though there is no significant correlation between yield and stand, would it be advisable to adjust the yields on stand even though no appreciable variance is evident for stand? Further, would this increase the efficiency of the experiment and reduce the standard error so that the three means approaching significance would be significant? The necessary parts of the analysis of variance concerning data adjusted for stand, computed by the short method, already described are presented in columns 9, 10, and 11 of table 12.

The adjustment of yield on stand has increased the variance due to residual error. There is an increase in the treatment variance accompanied by a slight increase in the size of F for treatment, but F for treatment remains insignificant. However, the slight increase in the size of the standard error, the increases in adjusted yields for high-yielding treatments, and decreases in low-yielding treatments brought about significant differences between nine means. The mean yield of the check plot was decreased, and the mean yields of two of the treatments which approached significance before correction now show a difference which exceeds the necessary ±3.59 tons for significance. There are now 4 treatments out of the 11 which exhibit significant differences over the check which may be attributed to fertilizer treatment. There are, however, no significant differences between any of the five high-yielding treatments.

The example given above demonstrates that it is possible to have significant differences between treatment means when F itself is not significant and also that adjustment of yield on stand as carried out here slightly increased the efficiency of the experiment. This often happens when there are such large differences between the extreme yields.

This is presented hereby as a statistical example and the experimental worker would hardly be justified in drawing any final conclusions from such data.

SIGNIFICANCE BETWEEN YIELD MEANS BEFORE ADJUSTING—NO SIGNIFICANCE AFTER ADJUSTING

Another example is presented where the yield treatment F is significant, the stand F approaches significance but, with one exception, none of the means show significant differences when compared with the check. These data are taken from the 1937 fertilizer-placement tests on Refugee beans grown for canning (table 13). The correlation coefficient for treatment is significant with a value equal to 0.913 but the r for error is 0.285 which for 17 degrees of freedom is not significant. The analyses of variances for the original data and the adjusted data are presented in table 14. The parts necessary for testing differences of adjusted yield means are given in columns 9, 10, and 11 of table 14.

TABLE 13.—*Yield in pounds per plot and stand for 1937 Refugee bean fertilizer-placement experiment*

Treatment	Yield and stand of plants per plot							Replication totals	
	B-1	B-2	B-3	B-4	B-5	B-6	B-7	Yield	Stand
Replication 1									
Yield	11.6	12.2	3.9	10.3	7.9	11.3	9.3	66.5	
Stand	105	100	55	99	92	97	89		637
Replication 2:									
Yield	12.5	14.2	9.2	10.6	12.2	14.3	13.5	86.5	
Stand	100	103	108	103	97	91	100		702
Replication 3.									
Yield	11.6	12.1	8.2	12.5	12.6	11.4	10.5	78.9	
Stand	112	101	80	99	108	107	98		705
Replication 4.									
Yield	11.2	9.7	9.9	10.2	10.3	10.5	8.0	69.8	
Stand	109	97	87	91	108	108	106		706
Mean yield	11.7	12.1	7.8	10.9	10.8	11.9	10.3		
Adjusted yield means	12.0	12.2	8.4	10.9	10.9	12.1	10.3		

TABLE 14.—*Analysis of variance and covariance for 1937 Refugee bean fertilizer-placement experiment* [1]

Source of variation	Degree of freedom	X=Stands		(XY)	Y=Yield		Correlation coefficients	Errors of estimate of yield		
		Sum of squares	Variance	Sum of products	Sum of squares	Variance		Sum of squares	Degree of freedom	Variance
Total	27	3,495		394.25	126.3					
Between replication means	3	487	162.3	81.15	35.1	11.7	0.621			
Between treatment means	6	1,342	223.7	239.55	51.2	8.5	[1].914		6	
Error	18	1,666	92.6	73.55	40.0	2.2	.285	36.8	17	2.16
Treatment plus error	24	3,008		313.10	91.2			58.6	23	
Treatment								21.8	6	3.65
F for treatment			2.42			[2] 3.86				1.69
b for error					0.0441					
Standard error						1.48				1.47

[1] Significant to 1-percent level [2] Significant to 5-percent level

That the variance for replication is significant before adjustment is clearly evident from table 14. Adjustment slightly reduced the residual error variance and the standard error, but it likewise reduced the treatment variance. The latter reduction was sufficient to reduce the significance of treatment *F*. There is an apparent reduction in efficiency if one considers only the lowered treatment variance. This was caused by correcting treatment No. 3 in which the fertilizer was placed below the seed, which very materially reduced the stand. This reduction in stand was very clearly evident in the field. If this treatment were corrected for stand and the yield computed from the regression equation, would this plot still be significantly lower? This was done; the mean for B–3 was not significantly less than the mean for the check (B–4). The mean of B–3 was significantly less than B–1, B–2, and B–6 after adjusting. It was significantly different from all the other means before adjusting. The variance for treatment yields is not significantly different from the variance for error after adjustment. Adjusting for stand reduced a significant *F* to a nonsignificant *F*; hence the differences in the beginning were due to differences in stand.

SUMMARY

The analysis of covariance between yield of marketable ears and stand of plants is presented for 26 hybrid strains and 1 open-pollinated strain of sweet corn. The yield data for five plots were lost, and a method of estimation for these missing plots is given. The correlation coefficient for error exceeds the 1-percent level, and an equation is given by which it is possible to adjust the yields on stand for each of the 108 plots.

A short method of deriving an analysis of variance for adjusted yields without the necessity of actually adjusting individual plot yields is presented. That this method is mathematically correct is substantiated by the derivation of essentially identical analyses of variance by both methods. The efficiency was increased by adjusting yields.

Another example is presented of maize planted in a 10 by 10 Latin square in which there was a significant residual correlation between yield and stand. The efficiency of the experiment, however, was not increased by adjustment of yield on stand in this case.

Data are presented for a fertility experiment with tomatoes grown from transplants. In this case treatment variance and the correlation coefficient between yield and stand were not significant, yet the efficiency gained in adjusting yield on stand is indicated when the adjusted treatment means are compared with the check (no treatment).

Additional data are presented, however, from a fertilizer placement test with Refugee beans. In this case the value of *F* for yields was not significant after adjusting for stand.

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A CRITICAL STUDY OF TECHNIQUE FOR MEASURING GRANULATION IN WHEAT MEAL[1]

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INTRODUCTION

Meal granulation, or degree of particle fineness, is regarded as an important component of wheat quality and is of considerable interest to the miller, cereal chemist, geneticist, and plant breeder. A simple method, therefore, of accurately measuring meal granulation would greatly facilitate a more thorough study and analysis of this character. Although a number of studies dealing with flour and meal granulation have been reported, few data are available to show the steps in the procedure used in establishing a satisfactory technique. In a preliminary paper Cutler and Brinson[2] described a method for measuring granulation of whole wheat meal. Further studies have been made with a view to perfecting and standardizing this method. This paper, therefore, presents data emanating from a systematic study of the steps in the procedure evolved in establishing a suitable technique for measuring meal granulation. New equipment and the nature of meal fractions are also described.

REVIEW OF LITERATURE

Several reports have been published in which separations of flour or meal particles of varying degrees of fineness were obtained by sifting on various sizes of bolting cloth and sieves. Since most of these are concerned with the chemical composition and baking quality of the various-sized fractions, only the more pertinent papers will be discussed.

Balakshin[3] used a farinogranometer to determine the relative coarseness of semolina, wheat flour, and buckwheat flour. Ten grams of the product were placed on the nest of sieves and sifted by hand. The fractions collected on the sieves were placed in a series of compartments forming columns from which a coarseness curve was plotted. In addition, cumulative coarseness curves were made from which it was possible to determine the relative coarseness of products by comparing the rectangles upon which the curves appeared.

According to Bailey,[4] "the simplest method of determining the granulation of flour involves sifting a weighed quantity, say 100 grams, through a series of bolting silks nested one above the other in order of increasing coarseness."

Kress[5] performed the granulation test by sifting 100 g of flour for 8 minutes in a seven-sieve experimental sifter. The eight fractions that

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[1] Received for publication May 9, 1938.

[2] CUTLER, G. H., and BRINSON, G. A. THE GRANULATION OF WHOLE WHEAT MEAL AND A METHOD OF EXPRESSING IT NUMERICALLY. Cereal Chem. 12: 120-129, illus. 1935.

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remained on each sieve and pan were collected, weighed, and the percentage of each calculated.

Cutler and Brinson [6] were the first to use wheat meal instead of flour for measuring the degree of particle fineness or granulation of strains and varieties of wheat. The relative granulation of the meal was determined by sifting 50 g of wheat meal for 1 hour in a two-sieve Ro-Tap. The weight of the meal on each sieve, as well as that in the pan, was used in calculating the granulation number for each sample. Likewise, Brinson [7] reported correlation coefficients of +0.90 and +0.83 in the 1931 and 1932 samples, respectively, between the meal in the pan and the "granulation number."

Fifield et al.,[8] using the method suggested by Cutler and Brinson [6] with modifications in sieve size, grinding, and method of expressing the results, found wide differences in degree of meal fineness in wheat varieties grown in the western region.

MATERIALS AND METHODS

In the investigations undertaken to study technique, three varieties of wheat having a wide range in meal granulation were used. The varieties were, in the order of increasing particle fineness, Michikof, Michigan Amber, and Purdue No. 1. Two thousand grams of sound, clean, normal wheat of each variety were prepared and stored in tight galvanized boxes. Representative samples for the study of each step in technique were obtained by reducing the 2,000-g lot in a Boerner sampler to an appropriate size. All steps in the procedure were studied with both medium and fine wheat meal, ground with the Labconco mill set at F and XF, respectively.

Moisture content of the wheat varied from 9.6 to 10.1 percent. From 12 to 32 replications were made in appraising each variable involved in the procedure.

Except for the variable under investigation the following method was used: 50 g of wheat were ground with the Labconco mill and placed in an airtight glass bottle. From this lot representative 2.5-g samples were placed on the top sieve and sifted for 1 hour in the granulometer, illustrated and described in this paper. The material passing through the finer sieve or into the pan was weighed and expressed in percent of the total, and designated as particle-size index. The higher the index the finer the sample, and the lower the index the coarser the sample. All tests involving any one step in the technique were completed within 6 days.

In tests comparing the two methods commonly used in designating meal granulation, 197 F_2 hybrids originating from a Michikof × American Banner cross and selected at random, were used. These hybrids furnished samples that not only possessed a wide range in degree of meal fineness, but were also suitable for statistical analysis.

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[6] CUTLER, G. H., and BRINSON, G. A. See footnote 2.

[7] BRINSON, G. A. THE RELATION OF GRANULATION OF WHOLE WHEAT MEAL TO "QUALITY" IN WHEAT WITH SPECIAL REFERENCE TO THE INFLUENCE OF FERTILIZER TREATMENT. 103 pp., illus. 1933. (Thesis submitted for M. S. degree, Purdue Univ.)

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Coefficients of correlation and differences necessary for significance between two samples or means were calculated to aid in the interpretation of the data.

DESCRIPTION OF EQUIPMENT

In developing new equipment for meal-granulation determinations, particular emphasis was placed upon its suitability for testing a large number of samples and using small amounts of wheat meal as well as for assuring accurate and dependable results. Such equipment, if available, would greatly aid the plant breeder and geneticist in studying the relative meal fineness in wheat varieties and hybrids. A large number of preliminary experiments were conducted in which various types and sizes of motors, speeds of sifting, width of sieves, agitating devices, etc., were used. As a result of these tests, the granulometer shown in figure 1 was developed.

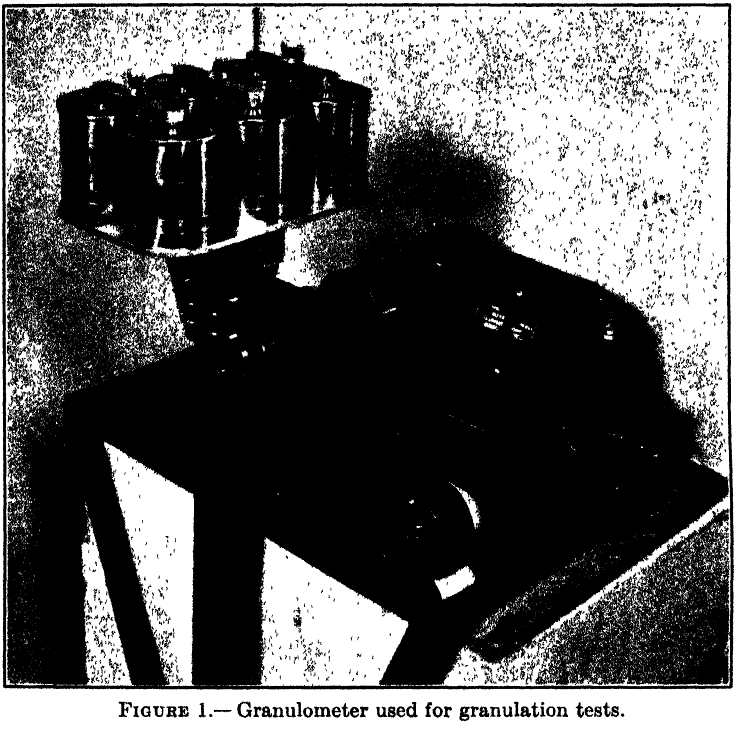

FIGURE 1.— Granulometer used for granulation tests.

The granulometer is of simple, sturdy construction mounted on rubber floor cushions on a concrete block. Six nests of sieves are held firmly to the sieve carriage by bolts with wing nuts. Each nest of sieves includes the following: cover, two 2¼-inch sieve frames fitted with United States standard 60- and 270-mesh wire cloth, and a pan. Passage of the material through the sieves is accomplished entirely by the force of gravity. The sieve carriage rotates in a vertical plane as a result of the eccentricity of the driving shaft, and is given a sudden jarring by a cam mounted on the outer end of the eccentric shaft. The impulses are generated by a 1/20-horsepower induction motor with built-in reduction gear which gives a speed to the main shaft of 216 revolutions per minute. A time switch is mounted on the base of the shaker. The granulometer is not only self-contained and cheap, but permits the testing of as many as 36 to 42 samples per day.

NATURE OF MEAL GRANULATION

It is a matter of common knowledge that flours derived from different classes and types of wheat differ in respect to granulation. Cutler and Brinson[9], using wheat meal, not only showed that granulation of the meal varied widely among different classes, but from a study of a large number of commercially grown soft and hard wheat varieties they demonstrated that bread flours originate from varieties that grind into a coarse meal, while pastry flours come from varieties that produce a fine meal.

Although considerable work has been reported on meal granulation, no information was found that showed the exact shape or form and nature of meal particles. In order to study the nature of the particles found in wheat meal, the meal fractions collected on top of the 60- and 270-mesh sieves and in the pan have been examined under the microscope. Studies were made with meal fractions representing varieties in the hard and soft wheat classes and with samples ground to varying degrees of fineness. In figure 2 are illustrated meal particles varying in size, shape, and angularity, of the three fractions originating from hard and soft wheats ground on the Labconco mill set at XF.

An examination of the individual fractions shown in figure 2 reveals a distinct difference in shape and angularity of the granules in the two samples. The granules in the hard wheat sample (*A*, *B*, and *C*) are characterized by sharp angular edges, while those in the soft wheat sample (*D*, *E*, and *F*) possess dull edges with rounded corners. The hard wheat sample is made up of gritty, vitreous particles, while those in the soft wheat are soft, starchy, and mealy in appearance. In addition to the great variations manifested in the meal granules, the proportion of meal collected on each sieve and pan varied greatly in the two samples. In the hard wheat, Michikof, 65 percent of the meal remained on top of the 60-mesh sieve, 25 percent on the 270-mesh sieve, and only 10 percent passed through the finer sieve and into the pan, whereas, in the soft wheat, Purdue No. 1, these proportions were 52, 28, and 20 percent, respectively. Moreover, flour milled from Michikof is of the coarse granular character usually associated with hard wheat flour, while that from Purdue No. 1 is soft, velvety, and smooth, characteristic of soft wheat flour.

EXPERIMENTS IN TECHNIQUE

The experiments dealing with technique were conducted over a period of 2 years, during which time more than 2,000 samples were sifted. The following steps in procedure were studied: (1) Numerical expression of meal granulation, (2) variation between tests, (3) variation among sieve sets, (4) duration of sifting, (5) size of sample, (6) amount of wheat ground, (7) speed of sifting, (8) moisture content of wheat, (9) age of meal, (10) fineness of grinding, and (11) experimental error in the test.

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[9] CUTLER, G. H., and BRINSON, G. A. See footnote 2.

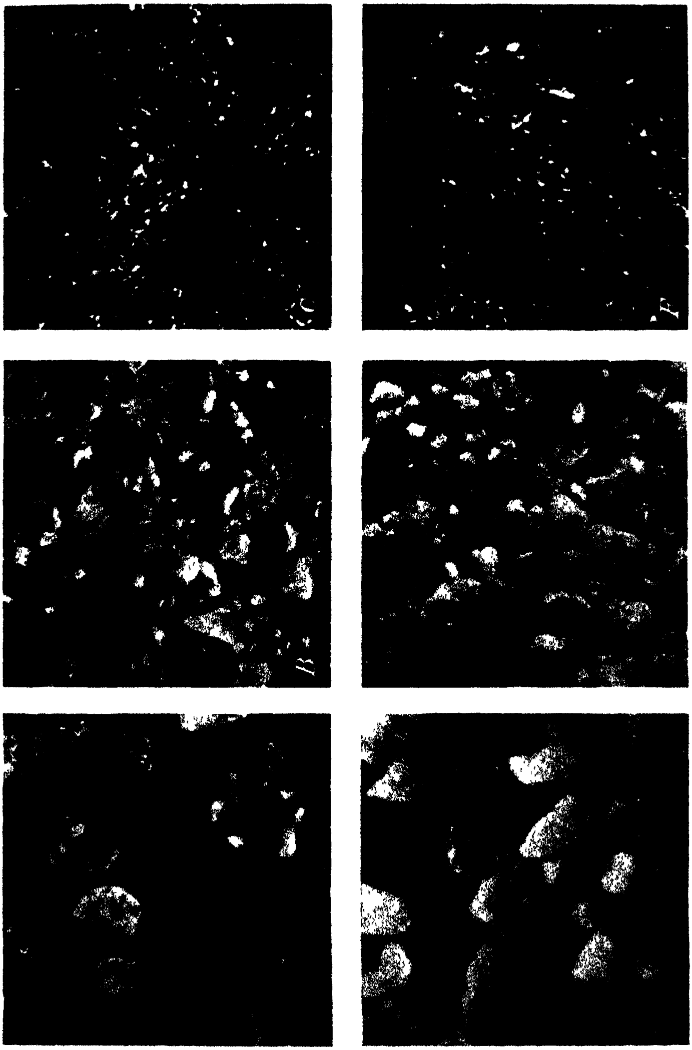

FIGURE 2.—Photomicrographs of coarse (*A* and *D*), medium (*B* and *E*), and fine (*C* and *F*) fractions of wheat meal. Fractions *A*, *B*, and *C*, representing a hard wheat, and *D*, *E*, and *F*, a soft wheat, illustrate the relative size, shape, angularity, and nature of particles or granules. × 20.

A large number of preliminary tests were conducted in which the various steps in procedure were tested in order to obtain information for carrying out the further tests which formed the basis for establishing a standard technique for measuring wheat meal granulation.

Only the data from these later experiments are reported. In presenting the data, averages of the three varieties used are reported, except in cases in which the individual varieties did not react uniformly to the particular variable under investigation.

NUMERICAL EXPRESSION OF MEAL GRANULATION

Obviously, there are many advantages in numerically designating the fineness of a given wheat meal. Two methods of expressing granulation results have been reported; namely, (1) granulation number in which all fractions are used in its calculation,[10] and (2) percentage of material that passes through the finest sieve or into the pan.[11] In these studies a comparison was made of the two methods. Samples of meal from 197 F_2 hybrids of Michikof×American Banner, selected at random were used. The samples varied greatly in degree of fineness. The relation between granulation number and percentage of material in the pan, as well as the interrelation between the proportions collected on the 60-mesh sieve, 270-mesh sieve, and pan are shown in the following tabulation:

Correlations between—	*Coefficient of correlation and standard error*
Meal in pan and granulation number	$+0.95 \pm 0.007$
Meal in pan and meal on 270-mesh sieve	$+.51 \pm .053$
Meal in pan and meal on 60-mesh sieve	$-.97 \pm .004$
Meal on 270-mesh and on 60-mesh sieves	$-.82 \pm .024$

The very high positive coefficient of correlation obtained between the percentage of material in the pan and granulation number indicates that the two methods are equally useful and efficient in numerically designating the relative fineness of wheat meal. However, inasmuch as fewer weighings are required and the subsequent calculations are greatly simplified, it was decided to use the percentage of material passing through the finer sieve or into the pan as an index of meal granulation. The percentage of the finest fraction was designated "particle-size index," and is used throughout this paper.

In comparing the relationship of the various proportions of coarse (on 60-mesh sieve), medium (on 270-mesh sieve), and very fine (pan) particle fractions resulting from the sifting tests of 197 hybrid samples, coefficients of correlation were calculated. The data show the following: (1) Increases in the proportion of very fine separations associated with increases in amount of medium-size fractions, or vice versa, (2) negative relation between the amounts of very fine and coarse meal fractions, and (3) negative relation between the medium and coarse particle fractions.

VARIATION BETWEEN TESTS

In determining the amount of variation that exists between different sieving tests, 16 separate tests were conducted in which medium and finely ground wheat meal were used. Each test included duplicate 2.5-g samples of Michikof, Michigan Amber, and Purdue No. 1, sifted for 60 minutes. The data obtained from each test were averaged and are shown in table 1.

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[10] BRINSON, G. A. See footnote 7.
CUTLER, G. H., and BRINSON, G. A. See footnote 2.

[11] BRINSON, G. A. See footnote 7.
FIFIELD, C. C., BODE, C. E., BAYLES, B. B., HAYES, J. F., WEAVER, R., and CHRISTIE, A. See footnote 8, both references.

The results from the different sieving tests are very consistent. An experimental error of less than 1 percent was obtained with either medium or finely ground meal. The variability of this factor is smaller than that usually found in laboratory and chemical tests, and should have little effect on the end results.

TABLE 1.—*Variation between different sieving tests* [1] *with medium- and finely-ground meal from three varieties of wheat*

Test No.	Particle-size index of meal		Test No.	Particle-size index of meal	
	Ground medium	Ground fine		Ground medium	Ground fine
	Percent	*Percent*		*Percent*	*Percent*
	10.9	18.3	6	10.8	18.4
	10.9	18.7	7	11.0	18.5
	10.8	18.6	8	10.7	18.7
	11.0	18.6	Standard deviation	.11	.15
	10.9	18.7	Coefficient of variability	.98	.82

[1] 12 samples used in each test.

VARIATION AMONG SIEVE SETS

Since six different sets or nests of sieves are employed on the carriage of the granulometer in determining the particle-size index of the meal, a study was made to determine the variation among the sieve sets and their location on the carriage. In studying this variable, 18 separate tests were made with medium and finely ground meal from three varieties of wheat. The data obtained are shown in table 2.

Small differences were found in the results obtained from the six different sets of sieves used in determining meal granulation. With medium ground meal the coefficient of variability was 0.92, while among sets in the fine meal it was 1.42 percent. No consistent relation was obtained between sieve sets and particle-size index either in the individual determinations or in the average of several tests.

DURATION OF SIFTING

To determine the duration of time of sifting needed for complete or nearly complete separation of the finest fraction, 2.5-g samples of meal were sifted for 15, 30, 45, 50, 60, 75, and 90 minutes in the granulometer.

TABLE 2.—*Variation in sieving tests* [1] *made with six different sets of sieves when medium and finely ground wheat meals were sifted simultaneously in the granulometer*

Sieve set No.	Particle-size index of meal		Sieve set No.	Particle-size index of meal	
	Ground medium	Ground fine		Ground medium	Ground fine
	Percent	*Percent*		*Percent*	*Percent*
1	11.0	17.6	5	10.8	17.2
2	10.9	17.6	6	11.0	17.0
3	10.8	17.2	Standard deviation	.10	.24
4	10.8	17.2	Coefficient of variability	.92	1.42

[1] 18 samples used in each test.

The average data obtained for the three varieties are shown in table 3.

TABLE 3.—*Influence of duration of sieving process upon the percentage of material sifted through a 270-mesh sieve from medium and finely ground wheat meal* [1]

Minutes sifted	Particle-size index of meal [2]		Minutes sifted	Particle-size index of meal [2]	
	Ground medium	Ground fine		Ground medium	Ground fine
	Percent	*Percent*		*Percent*	*Percent*
15	7.9	11.8	60	10.9	18.9
30	9.3	16.7	75	10.9	19.2
45	10.1	17.9	90	11.2	18.9
50	10.3	18.2			

[1] 12 samples used in each test

[2] Difference necessary for significance between two means, odds 19:1=0.37.

The data in table 3 show a gradual increase in the percentage of material passing through the finer sieve as the time of sifting was increased from 15 to 60 minutes. No significant differences were obtained between samples sifted for 60, 75, and 90 minutes. Consequently, in all comparative granulation tests, samples of wheat meal were sifted for 60 minutes in the granulometer.

SIZE OF SAMPLE

In the development of a suitable technique for measuring meal fineness special attention was given to the possibility of testing small samples. A series of experiments was conducted in which 1-, 2-, 2.5-, 3-, 4-, 5- and 10-g samples were sifted for 60 minutes in the granulometer. The average results obtained from medium and fine meal of three varieties are reported in table 4.

The data show no significant difference between the results obtained with 1-, 2-, 2.5-, and 3-g samples. In the larger samples, especially the 10-g sample, complete separation of the finest fraction was not accomplished during the 60-minute period of sifting. In all regular tests, the 2.5-g sample has been adopted as standard, because of the ease in sampling, reliability, and suitability for testing a few grams of wheat.

TABLE 4.—*Influence of size of sample* [1] *of medium and fine meal on completeness of separation of finest meal fraction*

Meal (grams)	Particle-size index of meal [2]		Meal (grams)	Particle-size index of meal [2]	
	Ground coarse	Ground fine		Ground coarse	Ground fine
	Percent	*Percent*		*Percent*	*Percent*
1	10.7	17.4	4	9.9	17.6
2	10.7	17.5	5	10.0	17.7
2.5	10.6	17.6	10	10.0	17.3
3	10.5	17.8			

[1] 12 samples used in each test

[2] Difference necessary for significance, 0.37.

AMOUNT OF WHEAT GROUND

From preliminary trials it was found that the quantity of wheat ground influenced the degree of fineness of the resulting wheat meal. To determine the effect of the amount of wheat ground on the percentage of the finest fraction or particle-size index, tests were made in which 5-, 10-, 15-, 25-, and 50-g lots of wheat were used. Separate lots of each sample size were ground with the Labconco mill set at F and XF to represent the medium and fine degrees of granulation. Since the varieties used did not react uniformly to the different amounts ground, the data for the three varieties are reported separately (table 5).

It will be noted that the size of the wheat sample ground greatly influenced the degree of particle fineness in the three varieties studied. In the soft wheat varieties Michigan Amber and Purdue No. 1, the larger the size of sample ground the finer was the meal, and the smaller the sample, the coarser the meal. With the hard wheat variety Michikof, on the other hand, the larger the quantity ground the coarser was the meal. Since the amount of wheat ground with the Labconco mill greatly affects the fineness of meal, it is necessary to grind samples of uniform size in all comparative tests. A 10-g-sample is recommended. It may be that the 10-g sample will not prove best with all wheats and under all conditions. For the conditions of the present experiments, however, it serves as a basis for standardizing a suitable technique.

TABLE 5.—*Influence of size of sample*[1] *ground on the fineness of medium and fine meal from three varieties of wheat*

Wheat ground (grams)	Particle-size index of meal[2]					
	Ground medium			Ground fine		
	Michikof	Michigan Amber	Purdue No. 1	Michikof	Michigan Amber	Purdue No. 1
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
5	7.0	10.8	12.6	13.4	17.6	22.2
10	7.2	10.6	13.8	12.4	18.8	23.2
15	7.0	11.2	13.4	12.2	18.4	23.4
25	6.8	11.4	14.2	12.0	18.4	24.0
50	6.8	12.4	14.4	11.4	19.2	24.6

[1] 12 samples used in each test.

[2] Difference necessary for significance between 2 means, odds 19.1=0.68.

SPEED OF SIFTING

It was found, from preliminary trials, that a speed of at least 200 r. p. m. was necessary before a satisfactory sifting of meal was obtained. In later tests the speed of the shaft was 190, 200, 220, and 240 r. p. m. The data obtained are given in table 6.

The data show that, under the conditions of these experiments, complete or nearly complete separation of the finest meal fraction was accomplished at a rate of speed of 220 and 240 r. p. m. As a result of these tests, a speed of 216 [12] r. p. m. was selected in making the new equipment and was adopted as standard.

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[12] Standard equipment.

MOISTURE CONTENT OF THE WHEAT

In determining the effect of moisture content of wheat on granulation of the meal, wheat samples varying widely in moisture content were used. Fifty-gram samples of each variety for each condition were placed in a humidifier and a dehydrator respectively for various periods of time. Immediately after the samples were taken out and ground, moisture-content determinations and sieving tests on the resulting meal were made. The data are shown in table 7.

TABLE 6.—*Influence of speed of sifting upon the completeness of separation of the finest fractions from medium and finely ground wheat*[1] [2] *meal*

Revolutions per minute	Particle-size index of meal[3]		Revolutions per minute	Particle-size index of meal[3]	
	Ground medium	Ground fine		Ground medium	Ground fine
	Percent	*Percent*		*Percent*	*Percent*
190	7.9	11.0	220	8.4	14.7
200	8.4	12.7	240	8.5	15.1

[1] Data are not comparable with those in other tables since the moisture content, source of samples, and the equipment used were different.
[2] 12 samples used in each test.
[3] Difference necessary for significance, 0.37.

TABLE 7.—*Influence of moisture content of the wheat used for grinding upon the percentage of material collected in the pan after sifting medium and finely ground meal*[1]

Moisture in wheat (percent)	Particle-size index of meal[2]		Moisture in wheat (percent)	Particle-size index of meal[2]	
	Ground medium	Ground fine		Ground medium	Ground fine
	Percent	*Percent*		*Percent*	*Percent*
7.6	9.1	16.2	10.0	10.4	17.6
8.7	10.5	17.3	10.9	12.0	20.8
9.0	10.3	17.4	12.4	11.7	[3]16.7
9.6	10.3	18.1	13.3	12.3	[3]15.3

[1] 12 samples used in each test.
[2] Difference necessary for significance, 0.37.
[3] A layer of meal formed on part of the 270-mesh sieve preventing complete separation of the fine meal.

Under the conditions of these experiments, wheat samples possessing a lower moisture content ground into a coarser meal than those of higher moisture content. The meal from samples containing 12.4 and 13.3 percent moisture formed a solid layer on a portion of the 270-mesh sieve. This prevented complete separation of the finest fraction and resulted in a lower particle-size index. Since the moisture content of wheat greatly influences meal granulation, it is necessary, in all comparative tests, to use samples of uniform moisture content. Considering all factors, wheat samples containing from 9 to 10 percent moisture have been found most suitable for granulation studies.

AGE OF THE MEAL

To determine the effect of age of meal on the results from granulation tests, 50-g samples of each variety were ground to the desired fineness and stored in airtight bottles. At weekly intervals representative samples from each lot were subjected to the regular sieving test. The results with meal stored for 0, 7, 14, 21, and 28 days are reported in table 8. They show that, in general, as the age of the meal increased, the particle-size index decreased. No significant difference is shown between the results obtained from meal tested immediately after grinding and that stored for 7 days in a tight bottle.

TABLE 8.—*Influence of age of coarse and finely ground wheat meal upon the results obtained in successive sievings made at weekly intervals* [1]

Aging period after grinding (days)	Particle-size index of meal [2]		Aging period after grinding (days)	Particle-size index of meal [2]	
	Ground coarse	Ground fine		Ground coarse	Ground fine
	Percent	*Percent*		*Percent*	*Percent*
0	10.6	18.1	21	9.8	17.1
7	10.9	17.8	28	10.1	17.1
14	10.3	17.8			

[1] 12 samples used in each test

[2] Difference necessary for significance, 0.37.

FINENESS OF GRINDING

One of the main objectives in establishing a suitable technique for granulation studies was to emphasize the difference between hard and soft wheat samples. From preliminary trials it was observed that greater differences in granulation appeared between hard and soft varieties in finely ground wheat meal than in meal ground coarse. In order to obtain specific data on this point, sieving tests were conducted with coarse, medium, medium fine, and fine meal ground with the Labconco mill set at 1, F, between F and XF, and XF, respectively. The data for the three varieties, together with the amount of difference that exists between Michikof and Purdue No. 1, are given in table 9.

Great differences are noted in the degree of meal fineness caused by grinding the wheat coarse, medium, medium fine, and fine. The data show that the greatest differences between a hard and a soft wheat were obtained from the samples that were ground the finest. Since the difference necessary for significance between two individual samples is not much greater in the fine meal than in the coarser samples, and since there are obvious advantages associated with a greater difference between hard and soft heats, wheat ground into a fine meal was adopted and used in all regular tests.

TABLE 9.—*Influence of fineness of grinding of wheat samples upon the difference between results in sieving tests with meals from hard and soft wheats* [1]

Variety	Particle-size index of meal			
	Ground coarse	Ground medium	Ground medium fine	Ground fine
	Percent	*Percent*	*Percent*	*Percent*
Michikof	5.0	5.7	7.0	9.9
Michigan Amber	8.4	11.1	13.8	19.6
Purdue No 1	10.5	13.8	16.6	24.3
Difference between Michikof and Purdue No 1	5.5	8.1	9.6	14.4
Difference necessary for significance between 2 samples-odds 19:1	.60	.56	.54	.81

[1] 32 samples used in each test.

EXPERIMENTAL ERROR IN THE TEST

To determine the error that exists in the technique used, and the amount of variation that could be expected from sifting several hundred samples, 50 separate tests with meals from three varieties of wheat, ground medium and fine, were conducted over a period of 1 month. Variations caused by sampling, weighing, date of grinding, tests, sieves, and moisture content are involved. The data for each variety as well as the difference necessary for significance between two samples, in medium and fine meal, are shown in table 10.

A variation of about 5 percent is shown in the technique recommended for granulation studies of wheat meal. Although the wheat samples for this study were stored in a tight galvanized container, the moisture content in the wheat gradually decreased from 9.9 to 9.6 percent during the period of this investigation. As a result, the first samples tested possessed a somewhat higher particle-size index than those tested at the end of the period. Moisture content, therefore, caused a great part of the variability obtained in the 300 samples tested. The data show that differences of 1.24 for medium and 2.00 for fine meal are necessary for significance between two samples in comparing results obtained under conditions somewhat similar to those in this experiment.

TABLE 10.—*Experimental error in technique adopted for meal-granulation studies in tests of three varieties of wheat ground medium and fine* [1]

Fineness of grinding	Variety	Mean	Generalized standard deviation	Coefficient of variability	Difference necessary for significance between 2 samples: odds 19:1
Medium	Michikof Michigan Amber Purdue No. 1	6.0 12.1 14.4	0.53	4.91	1.24
Fine	Michikof Michigan Amber Purdue No. 1	10.4 19.1 24.1	.86	4.80	2.00

[1] 50 samples used in each test.

SUMMARY

Experiments dealing with a critical study of technique for measuring meal granulation were conducted over a period of 2 years during which time more than 2,000 samples were sifted.

A new apparatus, the granulometer, was developed, and the nature of meal fractions are illustrated and described.

Steps in the study of procedure included (1) numerical expression of meal granulation; (2) variation between tests; (3) variation among sieve sets; (4) duration of sifting; (5) size of sample; (6) amount of wheat ground; (7) speed of sifting; (8) moisture content of the wheat; (9) age of the meal; (10) fineness of grinding; and (11) experimental error in the test.

From the results obtained in a systematic study of each step in the procedure, the following technique for measuring meal granulation has been adopted: 10 g. of clean, sound, normal wheat, containing about 10 percent of moisture, are ground into a fine meal with the Labconco mill set at XF. Immediately after grinding, a 2.5-g sample is weighed and transferred to the larger sieve (60-mesh and 270-mesh sieves used) and sifted for 1 hour in the granulometer. The material that passes through the finer sieve or into the pan is weighed. The weight of this fraction is expressed as percent of the total and is designated as particle-size index. A low index indicates a coarse meal and a higher index indicates a relatively finer meal.

CAROTENE BALANCE AND BLOOD-CAROTENE LEVELS IN HEIFERS AND LACTATING DAIRY COWS, AND THEIR RELATION TO THE PRODUCTION OF OFF-FLAVOR MILK[1]

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INTRODUCTION

In the course of an investigation of oxidized flavor in milk, it was discovered that four cows were excreting in their feces more carotene[2] than they were consuming. The flavor of the milk from each of these cows was markedly and quickly improved by adding a carotene concentrate[3] to the ration.

Since Atkeson and his coworkers (*1*),[4] Fraps and Treichler (*10*), and Moore (*18*) had already shown that only a small fraction of the total carotene ingested appears in the milk, either as carotene or vitamin A, it was not altogether unexpected that large quantities of carotene should be excreted in the feces.

In view of the great losses in the metabolism of carotene, it seemed desirable to examine the nature and quantity of these losses under variable conditions. The relationship of the excretion of carotene in the feces of cows to carotene intake and to the concentration of carotene in the blood serum was studied. The results are presented in this paper.

Except for carotene and vitamin A secreted in the milk, these studies were balance trials. In a previous study, Atkeson and his coworkers (*1*) showed that when cows were stall-fed green rye only 0.086 percent of a daily intake of 3.5 g carotene was deposited in the butter, as carotene, and only 0.154 percent as vitamin A. From these figures one might conclude that a carotene balance of the type here presented would not be appreciably affected by the small amounts of carotene secreted in the milk either as carotene or as vitamin A. Harris and Ray (*14*) found that animals without adequate reserves of vitamin C would use a suddenly increased supply much more efficiently than would animals with larger reserves. It was totally unexpected that cows showing evidence of carotene deficiency or even cows with moderate reserves would excrete in their feces carotene from body stores. The analogy to vitamin C, and consideration from this standpoint of certain experiments on children and rats might, however, have suggested this possibility.

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[1] Received for publication June 8, 1938. Contribution No. 233, Department of Chemistry, and No. 123, Department of Dairy Husbandry. Some of the material in this paper was presented orally at the Rochester meeting of the American Chemical Society, September 1937.

[2] The word "carotene," as used in this paper, refers to the petroleum-soluble pigment obtained by the methods described for the determination of β-carotene (*11, 19*).[4] There is some evidence that other pigments may be included in such a classification.

[3] This concentrate was a special preparation made by Nutritional Research Associates, Inc., by absorbing on wheat bran and middlings, a vegetable-oil solution of carotene previously obtained by extraction with fat solvents.

[4] Italic numbers in parentheses, refer to Literature Cited, p. 353.

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REVIEW OF LITERATURE

Relatively little consideration has been given to the ability of the bovine to absorb and retain carotene. Such studies have, however, been conducted on people. Rowntree (*21*) found that children on a variety of diets lost in the feces 2 to 12 percent of the carotene ingested. Infants fed a diet very low in vitamin A potency excreted for several days a larger amount of the vitamin than they ingested. Vitamin A was not distinguished from carotene in this study.

According to some workers the absorption of carotene from the intestine depends upon the simultaneous presence of a suitable fat or oil. Wilson, Das Gupta, and Ahmad (*23*) found that carotene fed to the human in the form of raw carrots or cooked spinach was about 90 percent absorbed when the diet contained fat, but only 50 percent when fat was omitted.

Booth et al. (*5*) found that carotene in butter was less potent for rats than an equivalent amount of vitamin A.

Basu (*3*) reported that carotene administered to rats in olive oil was almost completely absorbed, but in arachis oil approximately 90 percent could be recovered. With the same dosage of carotene, the rate of growth depended upon the oil used as a solvent, being greatest when linseed oil was used and least when arachis oil was used, while mustard, olive, sesame, and coconut oils occupied intermediate positions as solvents.

Coward (*6*) on the other hand maintains that there is no difference in the power of animals to utilize either vitamin A or carotene when supplied to them in cod-liver oil, plant tissues, butters, or as concentrates in various vegetable oils, and that the causes of the differences in effectiveness of carotene dissolved in different oils must be sought elsewhere.

De (*8*), working with rats under varying conditions of feeding, found only 45 to 65 percent of ingested carotene absorbed. De points out that differences in body weights of the experimental animals, or in the amounts of fat, yeast, and minerals in the diets did not cause significant variations in the absorption of carotene.

Moore (*17*) found that the concentration of pigment in the feces fat of rats was 12 times the concentration of the carotene in the ingested palm oil. A most consistent feature of these experiments was the persistence of carotene apparently unchanged within the alimentary tracts of the animals.

Dutcher et al. (*9*) fed carotene (without mineral oil) to rats at six levels, varying from 0.001 mg to 0.006 mg daily. Carotene excreted in the feces was not proportional to the intake. A 500-percent increase in intake resulted in only approximately 50-percent increase in carotene recovered from the feces; the relative losses were greater at lower intakes.

Wilson, Ahmad, and Majumdar (*22*) fed 0.05 mg of carotene per day to rats previously depleted of vitamin A reserves. The average recovery in feces was over 50 percent when the carotene was given as a suspension in glucose solution, or with 5 percent of bile, or with 15 percent of meat.

To determine the efficiency of absorption of carotene, Davies and Moore (*7*) examined the feces and urine of rats which had been receiving approximately 8.2 mg of carotene in coconut oil daily for 34 days. The amounts found represented 5 to 10 percent of the ingested carotene. This was less than one-tenth the relative loss observed by Wilson et al., but since the intake was 160 times that of Wilson's animals, the smaller relative loss was still 16 times the total loss of the animals on the lower intake. In the six tests reported by Dutcher et al. and also in the two tests reported, respectively, by Wilson et al. and by Davies and Moore the higher levels of intake accompanied the larger total loss. A smaller relative loss, however, prevailed with each larger intake.

Guilbert and Hart (*12*) have shown that carotene, the principal pigment of beef fat, may be withdrawn from the adipose tissue during vitamin A privation without a coincident reduction of the fat reserve. They also concluded that carotene in the fat of cattle constitutes a significant part of the total vitamin A reserve. Moore (*18*) pointed out that, in the case of the cow on pasture, the daily output of vitamin A and carotene in the milk fat is very small in comparison with the amount of carotene available in the diet. Storage of vitamin A in the liver takes place, while a large excess of pigment must undoubtedly be excreted unchanged. He also observed that the vitamin A content of the body fat remains within normal limits in the face of a large dietary excesss of carotene.

Before presenting detailed results on the large amounts of carotene excreted, a few remarks regarding the reliability of the measurements may be appropriate. It is quite likely that 5 to 10 percent of the yellow pigment measured as carotene was xanthophyll which alcohol failed to remove (*16*). Such an error, however, should apply equally well to the analyses of the rations and the excreta. Even an unbalanced error of this magnitude would not change the significance of the results.

Of particular interest to this problem is the finding of pseudocarotenes or additional pigments soluble in petroleum. Quackenbush, Steenbock, and Peterson (*20*) found that under certain conditions xanthophylls may be converted into petroleum-soluble pigments. These workers isolated five new carotenoids, designated pigments A, B, C, E, and F, from alfalfa silage and acidified fresh alfalfa. Three of these, pigments A, B, and C, are epiphasic. The usual methods of carotene analysis failed to differentiate between carotene and pigments A, B, and C.

Wiseman, Kane, Shinn, and Cary (*24*) have reported irregularities in the absorption curves of such pigments and of the fractions separated chromatographically.

Kemmerer and Fraps[5] also found yellow pigments in the excrement of rats and chickens that had received feed containing practically no carotene. These coloring matters could not be separated from the carotene by the usual procedure for extracting carotene. The absorption curves of these pigments were very different from that of carotene even though they absorbed some light in the same region of the spectrum.

EXPERIMENTAL PROCEDURE

The animals used in the present study included 6 yearling heifers and 17 lactating cows. The breeds represented were as follows: Heifers—two Holsteins, three Jerseys and one Guernsey; cows—six Holsteins, three Ayrshires, four Jerseys and four Guernseys. The various rations provided a range of carotene intake from 0.0028 to 2.51 mg per kilogram of body weight or a total daily intake varying from 1.25 to 1,252 mg. Some winter rations included silage made from Atlas sorgo as a carotene carrier.

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[5] KEMMERER, A. R., and FRAPS, G. S. DIGESTIBILITY OF CAROTENE BY RATS AND CHICKENS. Presented before Div. Biol. Chem. at Dallas meeting of Amer. Chem. Soc. Abs. 29, B14. 1938.

Carotene supplements to the winter rations were provided either as dehydrated fresh green oats, or as the carotene concentrate previously mentioned. A summer ration which included green grass was changed during the test to an almost carotene-free ration of beet pulp and grain. The best and most strongly supplemented winter ration contained about two-thirds as much carotene as the ration which included some green grass or pasture. Unsupplemented good winter rations provided about one-eighth of this maximum. For several cows these rations were also supplemented to raise this ratio to about one-third. Poor winter rations supplying from one-fiftieth to one-tenth the maximum were fed for from 1.5 to 5 months. A minimum ration supplying only one-seven hundredth of the same maximum was fed for 2 weeks.

Spectrophotometric carotene determinations by the method of Guilbert (*11*) as modified at this station (*19*) were used throughout. Optical densities were determined at the proper wave lengths for the two maxima and the minimum of β-carotene and had, in all cases, very nearly the correct ratio. The variations in amount of carotene excreted, both from day to day for a given individual, and from one individual to another on the same ration, were much larger than was expected (table 1). Differences between groups, however, were large enough to be significant in spite of these variations.

Separate parts of each ration fed were collected for carotene determinations. The daily consumption of each of these parts was weighed for each animal and the carotene intake calculated from these measurements. Feces samples were collected from each animal for 24-hour periods, weighed, mixed, and sampled for immediate analysis. Samples of blood from the jugular vein were collected daily and centrifuged, and the resulting serum was used for carotene analysis.

It is possible that certain ingredients of some rations contained compounds or adsorbates of carotene which were not extracted by the treatments used, although the best known methods of extraction were used. While some excess of carotene outgo over intake could be explained by assuming errors in extraction, the larger ratios could hardly be accounted for in this way. The low blood-carotene levels that accompany the rations measured as poor in carotene indicate that there was at least some significance in these measurements.

In all studies of the effect of change of ration, the first measurements were made well after the time considered necessary for a complete change of the contents of the alimentary tract (*2*, *15*). Several repeated tests at longer intervals after changing the ration indicated that the minimum intervals were sufficient. The two intervals of feeding beet pulp need not be interpreted as conflicting with this conclusion.

RESULTS

Daily variations that may be expected for a group of typical dairy cows are shown in table 1. The rations, the levels of blood carotene, the total average daily carotene intakes, the intake per kilogram of body weight, and the average daily excretions in feces for all trials are recorded in table 2. Each value given in this table for output and for blood level is the average of determinations on 3 successive days.

The data in table 2 have been presented from the standpoint of the rations fed. The lowest level of carotene intake at which animals were maintained for sufficient time to establish a fairly constant rate of depletion (this excludes the depletion experiment 5, b and 5, c) was in trial 1 a. In this trial a group of heifers had been for 5 months on a ration of cottonseed meal and straw sprinkled with molasses. The average carotene ingested was 0.043 mg per kilogram of body weight, or a total of 12 mg daily. When silage was fed with this ration to another group of heifers (1, c), the average carotene intake was approximately five times as much as for the group that received no silage. The amount of carotene excreted, however, was only 3.2 times as much.

Also, when the carotene intake was suddenly and greatly increased the group on the lower level of carotene intake (1, b) responded more emphatically and efficiently than did the group which had received silage (1, d). The drop in the ratio of output over intake was much greater (210 to 82 percent) for the group without silage. The carotene balance became positive in the group without silage, whereas it was still negative in the group receiving silage, even though the latter had a slightly larger intake.

TABLE 1.—*Daily variations of feces weight, carotene output in feces, and blood carotene for three typical cows*

Cow No.	Weight	Day of test	Wet feces weight	Total fecal carotene	Blood[1] carotene per 100 ml of serum
	Kilogram		*Kilograms*	*Milligrams*	*Milligram*
483	386	First	18.8	78	0.140
		Second	21.8	81	.148
		Third	19.9	92	.128
		Average	20.2	84	.139
493	439	First	28.8	144	.536
		Second	19.2	108	.428
		Third	17.5	120	.420
		Average	21.8	124	.461
496	309	First	15.6	33	.080
		Second	17.8	29	.057
		Third	21.2	23	.032
		Average	18.2	28	.056

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[1] From jugular vein.

Lactating cows (2, a), fed cottonseed meal, straw, and molasses for 6 weeks, showed a much better utilization of carotene, and the blood carotene values were approximately twice as large (0.075/0.041) as those for heifers (1, a) which had received the same rations for 5 months. This difference was possibly due to the residual effect of the cows having received good-quality alfalfa for 40 days preceding the shorter period of straw feeding. After adding carotene concentrate to the rations of both groups, 1, b and 2, b, the levels of carotene intake and the ratios of output over intake were very similar. The blood-carotene level for the lactating cows (2, b) now, however, was

TABLE 2.—*Variations in the carotene content of the blood and feces of dairy cows on different rations*

Series	Trial	Ration used	Period on ration	Milk flavor [1]	Cow		Daily carotene intake		Daily carotene output, total	Ratio output intake	Blood [3] carotene per 100 milliliter of serum
					No [2]	Weight	Kilogram of body weight	Total			
						Kilogram	*Milligram*	*Milligram*	*Milligram*	*Percent*	*Milligram*
No. 1	a	Cottonseed, straw and molasses	5 months	(4)	144	405	0 037	15	22	147	0.027
					390	236	.047	11	27	245	.043
					391	257	.047	12	28	233	.039
					498	309	042	13	28	215	.056
				Average		302	.043	12	26	210	.041
	b	Same as 1a, plus 225 g of carotene concentrate daily.	2 weeks	(4)	144		212	86	85	99	.085
					390		.347	82	69	84	.097
					391		.368	95	63	67	.135
					498		.306	95	72	76	.175
				Average			.308	89	72	82	.122
	c	Cottonseed, straw, molasses, silage	5 months	(4)	147	400	.200	80	90	112	.175
					392	349	254	56	73	130	.098
				Average		374	.227	68	82	121	.136
	d	Same as 1c, plus 225 g of carotene concentrate daily.	2 weeks	(4)	147		.382	153	155	101	.281
					392		340	119	138	116	.151
				Average			.361	136	146	108	.216
No. 2	a	Cottonseed, straw, molasses	6 weeks, preceded by 40 days on alfalfa, corn, and bran.	Oxidized (except 109).	109	560	.064	36	61	169	.080
					249	471	.060	27	41	152	.080
					373	400	.068	27	25	93	.068
					464	423	044	19	25	132	.074
				Average		463	.059	27	38	137	.075
	b	Same as 2a, plus 450 g of carotene concentrate daily	2 weeks	Good	109		.322	181	157	86	.320
					249		.366	172	167	97	317
					373		.432	173	148	86	.302
					464		.388	164	141	86	.298
				Average			.377	172	153	88	.309
No. 3	a	Yellow corn, bran, alfalfa	6 weeks, preceded by ration 2a for 40 days	do	126	571	218	125	137	110	.263
					240	510	.147	75	110	147	.201
					378	378	195	74	84	113	.156
					483	386	231	89	84	94	.139
				Average		461	.198	91	104	117	.189

No. 4	a	Yellow corn, oats, bran, linseed meal, silage, alfalfa	5 months	do	112	624	.364	227	215	95	.317
					374	575	294	110	135	123	.418
					493	439	259	14	124	109	.461
				Average		479	.306	150	158	109	.398
	b	do	5 months	Oxidized	130	640	211	135	214	158	.477
					134	456	.298	136	126	93	.488
					242	498	.278	139	150	108	.445
					370	435	.279	122	123	101	.400
				Average		507	266	133	153	115	.452
				Average of 7.		495	283	140	155	112	.429
	c	Same as 4a, plus 0.9 kg of dehydrated oats daily.	2 weeks	Good	130		576	368	299	81	.658
					242		.744	371	261	70	.657
					370		814	355	261	74	401
				Average			.711	365	273	75	.572
	d	do	4 weeks	do	130		.578	369	369	100	.566
					242		.743	371	353	95	.585
					370		.811	354	304	84	.396
				Average			.711	364	342	93	.515
	e	Same as 4a, plus 1 8 kg of carotene concentrate daily.	2 weeks	do	134		1 53	697	544	78	.509
No. 5	a	Grain, alfalfa, silage, as in 3a, plus pasturage.	3 months	(5)	190	600	2.073	1,252	1,100	88	1 040
					486	450	2 511	1,130	980	87	1 420
				Average		525	2 292	1,191	1,040	87	1 230
	b	Linseed meal, molasses, beet pulp	1 week	do	190		0031	1 88	28 1	1,495	590
					486		0028	1 25	18 1	1,450	840
				Average			0029	1.56	23 1	1,470	.715
	c	do	2 weeks	do	190		0031	1 88	3.20	170	.246
					486		0028	1 25	2.25	180	.265
				Average			0029	1.56	2 72	175	.255

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[1] The milk flavors recorded here were part of a study by Beck, Whitnah, and Martin (4).
[2] The breeds are indicated by the herd numbers used. Holstein=100+, Ayrshire=200+; Jersey=300+; Guernsey=400+
[3] From jugular vein.
[4] Heifers not in milk.
[5] Not tested.

more than twice (0.309/0.122) that of the heifers (1, b). These results show that feeding histories for several months before tests are important in studies involving carotene metabolism, and that the maximum value may be attributed to the results only when the possible effects of factors other than current carotene intake have been considered.

When alfalfa with corn and bran was substituted for straw with cottonseed meal and molasses during a 6-week period (3, a) the carotene intake per kilogram of body weight was three times that of the straw-fed cows (3, a/2, a=0.198/0.059). For the alfalfa-fed cows the ratio of carotene output over intake differed but little from the ratio for straw-fed cows. The blood-carotene level, however, was more than twice as high, and the milk flavor was much better.

When cows receiving grain, alfalfa, and silage had their ration supplemented by the addition of 0.9 kg of dehydrated green oats daily (4, c), thereby more than doubling the carotene intake, great improvement in the utilization of carotene resulted. In 2 weeks the ratio of output over intake dropped from 115 percent to 75 percent, and the blood-carotene level increased from 0.452 to 0.572 mg. When the supplement was continued for 2 weeks longer a decrease in the efficiency of utilization occurred. The carotene excreted increased from 75 to 93 percent of the intake. The average blood-carotene level, however, only fell from 0.572 to 0.515 mg. A cow (4, e) receiving 1.53 mg of carotene per kilogram of body weight, or (about five times the intake of the cows receiving grain, alfalfa, and silage, and twice the intake of cows receiving the oats supplement (4, c), did not show a higher blood-carotene level nor did she utilize carotene more efficiently.

Cows under pasture conditions (5, a), and hence receiving a very much higher level of carotene (2.292 mg) than any of the others, had a positive balance and a remarkably high blood-carotene value (1.23). Cows on good winter rations, even with the largest amounts of dry carotene supplements, (4, c), reached a maximum blood level at an average value of 0.572 mg per 100 ml. It appears likely that prolonged feeding even at the highest carotene level used on winter rations would not have increased the blood-carotene level to that which was obtained under pasture conditions. It is entirely possible that other factors present in fresh green feeds may be responsible for what appears to be a more complete assimilation of carotene from pasture.

Although shortly after cows (5, b) were transferred from a summer ration to a winter ration their blood carotene was higher than the maximum produced with dry supplements, the higher value was rapidly declining and was not a maximum.

When cows which had been on a very high carotene intake from grass (5, a) were put on a ration practically devoid of carotene, the total fecal output of carotene dropped 98 percent the first week and 88 percent of the remainder during the second week. This final value does not appear to be a minimum, but it is insignificant compared with any other observed value. The blood-carotene, however, dropped only 42 percent the first week and 64 percent of the remainder the second. Even after 2 weeks on the carotene-free ration, the blood carotene was as high as that of many cows that had been receiving alfalfa and grain for 6 weeks. It would appear that the blood-carotene level maintains itself for some length of time after the animal has been placed on a carotene-free ration.

In agreement with findings from studies on rats, the total amounts of carotene excreted were lower at the lower levels of intake, but the ratios of output over intake were higher. All the eight ratios above 160 percent accompanied intakes of less than 0.150 mg per kilogram of body weight; and twelve of the fourteen ratios above 125 percent accompanied intakes of less than 0.210 mg per kilogram.

In most cases the classification of lactating cows from the standpoint of rations has also classified them from the standpoint of milk which would or would not develop oxidized flavor. The tests with intakes below 0.075 mg per kilogram (2, a) involved milk that developed oxidized flavor. All but one of the cows in this trial group and all the heifers with intakes below 0.075 mg per kilogram were excreting more than 130 percent of their carotene intake. In the 12 tests with intakes above 0.300 mg per kilogram (2, b; cow 112 of 4, a, 4, c; 4, d; and 4, e) all the cows produced good-flavored milk and the output-over-intake ratios were all 100 percent or less. For intake levels between 0.100 and 0.300 mg the relation to milk flavor, and the relation to output-over-intake ratio were irregular.

In two groups of cows receiving the typical winter ration (grain mixture, alfalfa, and silage) for 5 months, and having nearly the same carotene intake (0.306 and 0.266), the milk flavor was good in the case of one group (4, a) while the milk from the other group (4, b) developed oxidized flavor. The group producing milk that developed oxidized flavor had a slightly higher blood carotene value and slightly lower intake. While neither of these differences considered alone seems sufficient to explain the difference in milk flavor, the difference in ratio of blood carotene to intake level is large enough to suggest that at these levels of intake the maintenance of a high level for blood carotene competes with the production of good-flavored milk. These two groups (4, a and 4, b) had a much higher blood carotene level (0.398 and 0.452/0.122 and 0.216) than heifers which were at about the same carotene-intake level after 2 weeks feeding of a ration containing cottonseed meal, straw, and molasses (0.308 for 1, b and 0.361 for 1, d) supplemented with a carotene concentrate. This indicates either that the heifers had a lower capacity to metabolize carotene or that 2 weeks was not a long enough period to bring the blood carotene up to the level maintained by an equal intake on a different ration.

That the carotene-intake level was not the only factor determining the tendency of milk to develop oxidized flavor is also shown by the fact that the five lowest carotene intakes per kilogram of body weight among cows producing milk that kept a good flavor (109 of 2, a, and all cows of 3, a, average=0.171) were below the three highest intakes among those cows producing milk which developed oxidized flavor (4, b, except 130 average=0.285). The first group had lower blood carotene levels (average=0.168) than the second (average=0.444). For cows producing poor milk on either poor or good winter rations, a sudden substantial increase in carotene intake produced both a rise in the level of blood carotene and an improvement in milk flavor. Possibly the oxidized milk flavor represents a tendency of some cows to care for the needs of their own bodies before protecting their milk. On the other hand it may be that the milk developed oxidized flavor, and the blood carotene level was high because these cows were not efficient in transforming carotene into vitamin A. The former theory would more nearly fit the good flavor of milk from cow 109.

DISCUSSION

The daily carotene requirement of the bovine and other species according to Guilbert and Hart (*13*) is quite constant at 0.026 to 0.033 mg per kilogram of body weight. No evidence has been found that the physiological differences associated with lactation seriously affect carotene metabolism. In every case in this study, except that of cows receiving beet pulp and grain, the daily carotene intake was in excess of the minimum requirement. There is evidence, however, that many of the animals had less than an optimum supply of carotene. The frequent negative carotene balances, the existence of blood carotene levels far below those of cows on pasture, and the production of off-flavored milk which was corrected by feeding carotene supplements are such evidence.

Guilbert and Hart (*13*) also reported that the daily withdrawal of carotene from body stores when cows were on a vitamin-A-free ration was about 9 to 11 mg. From Guilbert's data (*11*) on carotene stores in body fat we might expect the straw-fed animals to maintain their measured carotene excretion for only 1 or 2 weeks. During the 6 weeks to 5 months preceding the tests both the output and the intake of carotene had probably been decreasing. The decrease of intake would be due to the slow decomposition of carotene in the rations. This loss might be proportional to the time of storage. The expected decrease of output would be due to the decreasing reserves and should decrease more nearly in a geometric ratio to time. It is likely then, that the excretion had decreased faster than the intake. It has been found (*17*) for rats that the intestines contain carotene several weeks after a carotene supplement has been withdrawn from an otherwise vitamin-A-free ration. While the intestines of cattle sometimes appear yellow, their carotene content is unknown. It may be assumed either that only a small fraction of the actual carotene intake was measured, that the bovine has some large unknown depot for the storage of carotene, or that something measured as carotene was produced within the animal. It is indeed fortunate that the relative absorptions at different wave lengths and the small minimum observed excretion satisfy so well tests proposed later by Wiseman et al. (*24*), and by Kemmerer and Fraps [6] to prove that the pigment was actually carotene.

SUMMARY

Spectrophotometric carotene determinations were made on rations, blood, and feces of cows on various rations with and without carotene supplements. The petroleum-soluble pigment obtained by the methods described for the determination of B-carotene was calculated as carotene. At levels of intake varying from 0.003 to 2.51 mg per kilogram of body weight, a relatively large amount of carotene was excreted in the feces. The average percentage of ingested carotene found in the feces of different groups varied from 75 to 1,470. The concentration of carotene in blood serum was found to vary greatly with the carotene intake and with the individual cow. The 3-day averages for carotene in blood serum varied from 0.027 to 1.42 mg per 100 ml.

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[6] KEMMERER, A. R., and FRAPS, G. S. See footnote 5.

Two groups of heifers were studied. Both received cottonseed meal and straw sprinkled with molasses. One group received, in addition, sorgo silage. The silage group receive five times as much carotene as the others, but excreted only 3.2 times as much. Supplements produced a much larger response in the nonsilage groups.

Cows which had received cottonseed meal, straw, and molasses for 6 weeks were compared with two other groups; one, the nonsilage-fed heifers, the other, a group of cows which received corn, bran, and alfalfa for 6 weeks following 6 weeks on the straw ration. The cows receiving cottonseed meal, straw, and molasses, showed better utilization of carotene and higher blood carotene levels than the heifers, and lower blood carotene levels than the alfalfa-fed cows. The two groups of cows, however, lost about equal proportions of their carotene intakes. The straw-fed cows responded to a supplement of carotene concentrate much better than the heifers. These results show that feeding histories for several months may be important in tests involving carotene metabolism.

The supplementing of a good winter ration of grain, alfalfa, and silage with carotene concentrate, dehydrated green oats, or fresh pasture grass showed greatly increased absorption of carotene in all cases. The most efficient utilization was observed 2 weeks after the dry supplements were first fed, but by 4 weeks the efficiency had dropped below that on fresh grass. It is entirely possible that factors in fresh grass, other than its carotene content, may be responsible for the better assimilation of carotene from the fresh grass.

When cows were transferred from a ration supplemented with pasture to a ration nearly devoid of carotene the fecal output of carotene dropped 98 percent the first week and 88 peicent of the remainder the second week. The remainder after 2 weeks is insignificant compared with any other carotene excretion observed.

Low carotene intakes were quite regularly associated with milk which developed oxidized flavor, while high carotene intakes prevented or remedied this defect. The relation of flavor to moderate intakes was irregular. There is some indication that, with moderate carotene intake, the maintenance of the proper level of blood carotene competes with the protection of good flavor in milk, The level of carotene intake was possibly not the only factor determining the tendency of milk to develop oxidized flavor.

The optimum level of carotene intake appears to be many times the minimum intake required to protect from recognized deficiencies. The finding that the ratio of carotene output to intake increases at lower intakes is in agreement with data which other workers have reported.

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THE ANATOMY OF SPRUCE NEEDLES[1]

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INTRODUCTION

In 1865 Thomas (*16*)[4] made a comparative study of the anatomy of conifer leaves and found that the structural variations exhibited by the different species warranted taxonomic consideration. Since that time leaf anatomy has become a fertile and interesting field of research. Nearly all genera of gymnosperms have received some attention, and the literature on this subject has become voluminous. A detailed review of the literature will not be attempted in this paper, since comprehensive reviews have already been published by Fulling (*6*) and Lacassagne (*11*). Fulling's paper contains in addition an extensive bibliography on conifer leaf anatomy.

Most workers in this field of research have confined their efforts to the study of the cross sections of needles. This is partly because longitudinal sections are difficult to obtain and partly because they present but little structural variation of value for identification. The workers who have studied both longitudinal and cross sections have restricted their descriptions of longitudinal sections either to specific tissues or to a few species of a large number of genera, and the descriptions, although comprehensive, leave much to be desired from the standpoint of detailed information and illustration.

Dorner (*3*) was perhaps the first to use sketches to augment keys to and descriptions of the native firs and spruces. His diagrammatic sketches portray the shape of the needles in cross section and the position of the resin canals. Durrell (*4*) went a step further and illustrated his notes on the North American conifers by camera-lucida drawings depicting the orientation and arrangement of the various needle tissues in cross section.

Since the development of technique for taking them, photomicrographs have largely supplanted drawings. Such photographs are valuable aids in identifying and recording species, as can readily be appreciated by reviewing the works of Harlow (*9*), Fulling (*6*), and Sutherland (*15*).

The pines (*Pinus* spp.) and the firs (*Abies* spp.) have been the subjects of most conifer leaf studies. A few authors, however, have directed their attention to the study of the foliar anatomy of the spruces (*Picea* spp.), among them Burnet (*2*), Bastin and Trimble (*1*), Hayata and Satake (*10*), Lacassagne (*11*), and Marco (*12*). The studies of these authors were based upon cross sections. In none of their papers appears a complete description of the internal structures of spruce leaves. Further, there is considerable disagreement among the writers

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[4] Italic numbers in parentheses refer to Literature Cited, p. 367

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concerning the shape of the needles in cross section, the number and position of resin canals, and the relative merits of various diagnostic characters.

It is the purpose of the present paper to describe in detail the internal structures of spruce needles as seen in both cross and longitudinal sections and to explain something of their functions. Where possible these structures will be illustrated by photomicrographs.

THE ANATOMY OF SPRUCE NEEDLES

For convenience the internal structures of a spruce needle may be divided into three general tissues—the dermal, the mesophyll, and the vascular. The dermal tissue embraces the epidermis and hypodermis. The mesophyll comprises the green, usually isodiametric cells which form the bulk of the needle. The vascular tissue comprises the endodermis, the transfusion cells, and the fibrovascular bundles. The resin canals are located in the mesophyll adjacent to the dermal tissue (pl. 1, *A*).

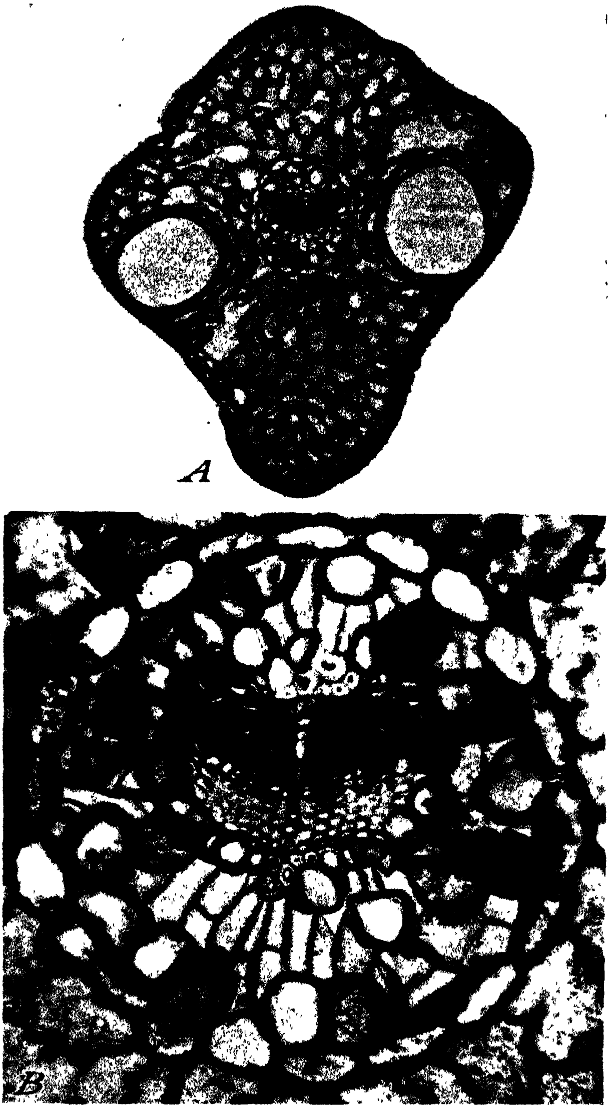
THE EPIDERMIS

The epidermis is a single peripheral layer of cells, continuous except for stomatal openings. The cells, which are irregular in shape and size (pl. 2, *A*), are firmly fastened to one another by the interlocking of their walls. They measure approximately two to three times as long as they are wide; are half as thick as wide; and are oriented with their long axis parallel to that of the needle.

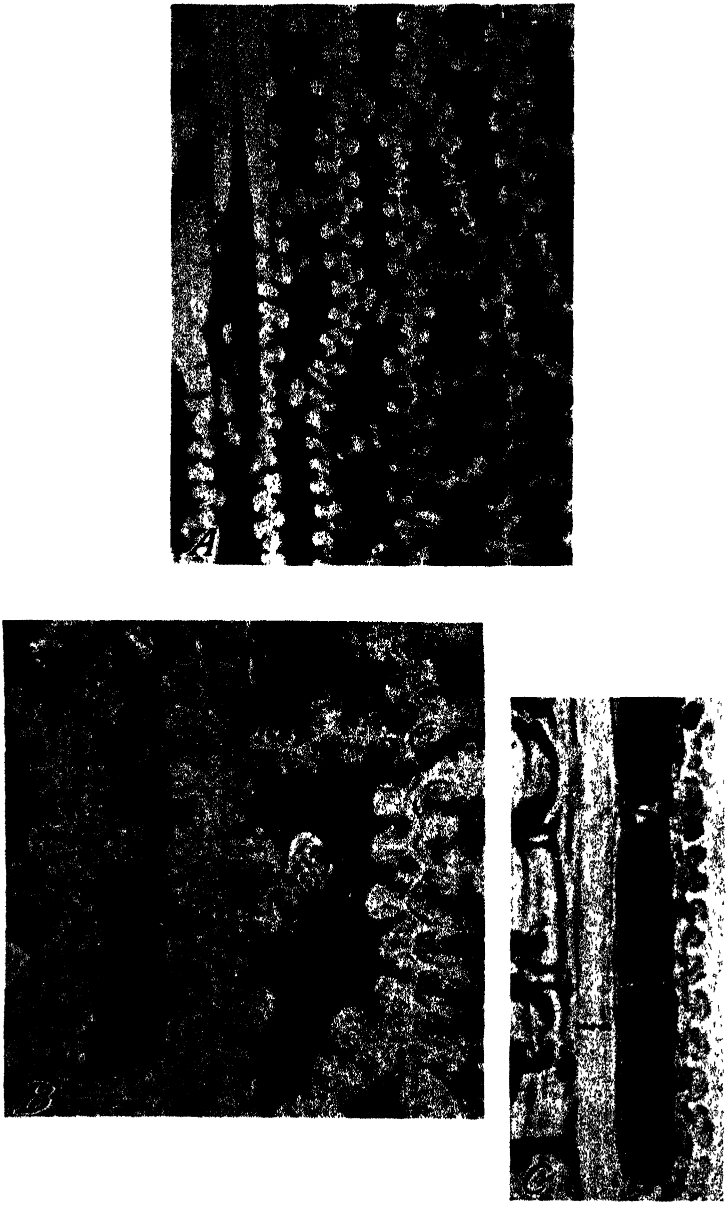
The lateral, cross, and inner walls of the cells appear equally and uniformly thickened. The outer walls, which arch outwardly, are in part considerably thicker than the other walls. The thickest portion of the outer walls is near the center of the arches. From this region the walls gradually become thinner toward their margins until they are of the same thickness as the lateral and cross walls.

The epidermal cells lock together in an unusual manner. As seen in plane section, the lateral and cross walls appear incised or mortised at regularly spaced intervals (pl. 2, *B*). The outer portion of the cell wall between the mortises is slightly gabled. Extending outward from the peak of the gable is a narrow, tenonlike projection of the wall. These projections or tenons are slightly wedge-shaped, with the outer extremities wider than the inner. The walls of adjoining epidermal cells are constructed in the same manner, but the tenons of one cell are opposite the mortises of another. Consequently the epidermal cells are held together by a modified dovetailed joint. This explains why the epidermal cells cling tenaciously together even after a prolonged treatment in Jeffrey's or Schultze's macerating fluid. The walls of *Picea breweriana* S. Watson, in addition to being mortised and tenoned, are infolded.

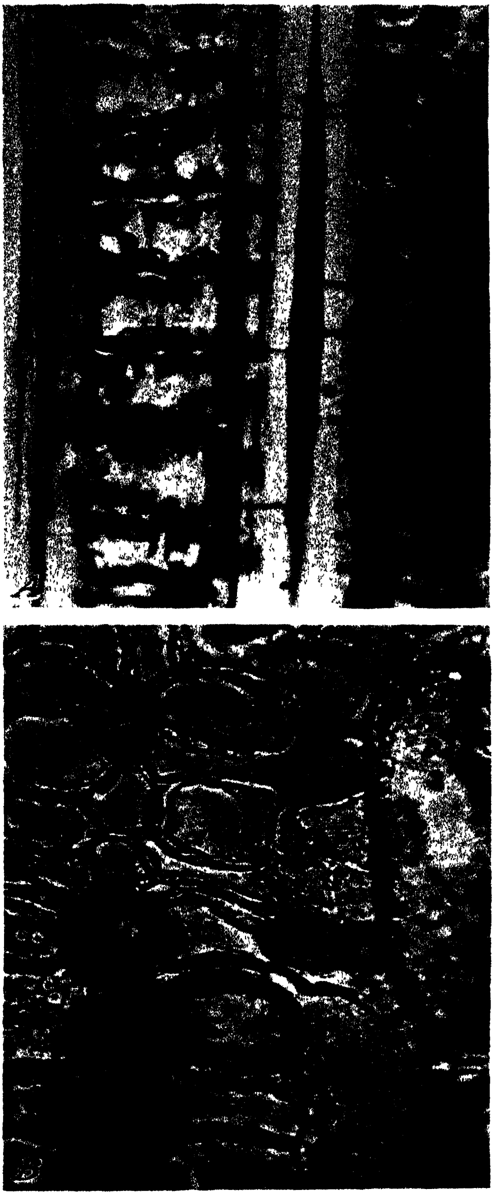
Around the periphery of the epidermal cells, immediately above and part of the cell wall between the mortises, are a number of toothlike projections which extend outwardly into the cuticle. The teeth, as seen in longitudinal sections, are variable in shape, ranging from square to mushroomlike. The latter type, which is the more common, also shows considerable diversification of crown forms. The crowns may be pyriform, hemispherical, or divided into rounded, hooked lobes. The teeth in *Picea breweriana* are accentuated by the infolding of the cell wall between the denticles (pl. 2, *C*).



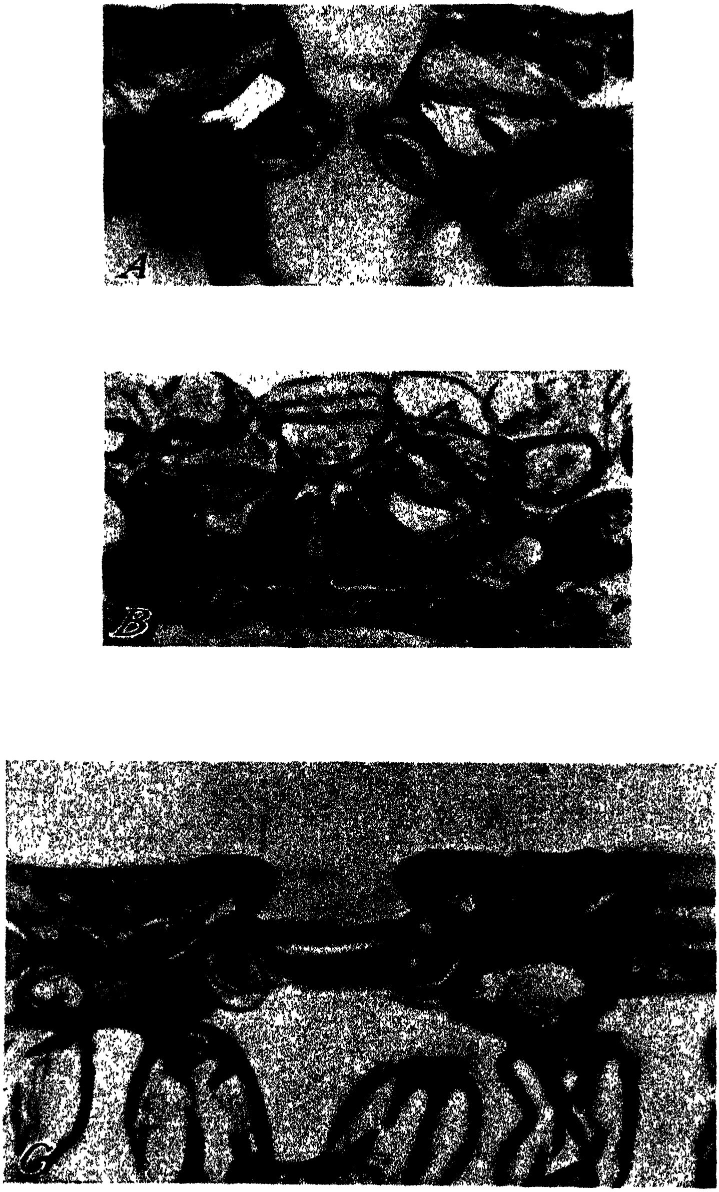
1, A typical cross section of a needle of *Picea engelmannii* (Parry) Engelm. Beginning at the circumference, the various tissues depicted are as follows: The outer peripheral row of cells is the epidermis; the second peripheral row of cells is the hypodermis; the interruptions in the epidermis and hypodermis are the stomata; within the hypodermis is the mesophyll; the ring of cells near the center of the needle is the endodermis; within the endodermis are the fibrovascular bundle and transfusion cells. Note the two large resin canals in the lateral angles of the needle. × 75. *B*, The central portion of a cross section of a *P. breweriana* needle showing the endodermis, transfusion cells, and fibrovascular bundle. The upper portion of the bundle is the phloem; the lower, the xylem. Note the strengthening cells or fibers above, below, and adjacent to the bundle. × 210.



A, Tangential section through the epidermal cells of *Picea breweriana* showing the structure of the cell walls, × 350; *B*, a portion of the same section at a higher magnification to show the method by which the cells are locked together, × 890; *C*, longitudinal section through the epidermal cells of *P. rubra* Link, depicting the teeth on the outer walls, × 890.



A, Longitudinal section through the dermal region of *Picea breweriana* illustrating the simple pits and fusiform lumen of a hypodermal cell (center); the infolding of the epidermal cell walls (right); and the chloroplasts in the mesophyll cells (left). × 440. *B*, Longitudinal section through the transfusion tissue of *P. breweriana* portraying the border-pitted tracheal cells and the thick-walled, simply pitted endodermal cell (right). × 400.



A, Cross section through a stoma of *Picea rubra* depicting the excessively thickened upper walls of the accessory cells and the two elliptical guard cells. Note that the upper walls of the guard cells are distinct from the lower walls. The section was made near the center of the stoma. × 890. *B*, Cross section of the stoma shown in *A* near the ends of the guard cells. Note that the guard cells have become right-triangular in shape. × 890. *C*, Longitudinal section through a stoma of *P. rubra*.

In cross section these structures appear as a double row of rounded wedges inserted between two epidermal cells. They do not usually protrude into the cuticle beyond the arch of the outer cell wall except in some species, e. g., *Picea breweriana* and *P. asperata* Masters. The denticulations become almost indistinguishable in plane sections, since they merge with the cell walls in the background. When discernible the teeth are oblong in outline.

The cross, lateral, and tangential walls of the epidermal cells contain simple pits (pl. 2, *C*) by means of which the cells communicate with one another and with cells of the adjacent hypodermis. Blind pits that lead to the cuticle can occasionally be detected in the outer walls. Feustel (*5*) intimates that the epidermal pits are always closed, but no evidence was found in the present study to support this contention.

The epidermal cells contain large, conspicuous nuclei and thin cytoplasmic layers. The nuclei are generally found near the centers of the cells and are held in position by a number of minute cytoplasmic threads. The cytoplasmic layers are very thin and are difficult to distinguish from the cell walls, to which they closely adhere. The vacuoles, which fill most of the cell cavity, frequently contain tanniferous substances that often impart a brownish tinge to the cell contents and render them opaque.

Overlying the epidermis and continuous except for the stomata is the cuticle. This waxy layer, although universally present, varies in thickness with different species. For example, in *Picea breweriana* the cuticle is as thick as the epidermal cells, while in *P. spinulosa* (Griffith) Henry, it is much thinner and barely distinguishable from the cell walls upon which it lies. However, the cuticle is always sufficiently thick to cover the epidermal teeth. According to Gauba (*7*) the cuticle consists of two layers. The outer layer appears as a homogeneous, tough, waxy film; the inner is composed of small, densely packed, waxy particles. These particles enclose the teeth and closely follow the contours of the outer walls of the epidermal cells. The structure of the cuticle can be seen readily in *P. breweriana*.

THE HYPODERMIS

Beneath and in contact with the epidermis lies the hypodermis. This tissue, like the former, is continuous except where interrupted by the stomata and resin canals. The hypodermis, however, is composed of libriform fibers and frequently is more than one cell thick, especially in the angles of the needles. The fibers are often septate; are long to very long and thick to very thick walled; and have numerous simple pits and narrow lumina. In cross section the lumina of the fibers, which appear isodiametric, vary in size. The variation is caused by the shape of the lumina. As seen in longitudinal section, the lumina are narrowly spindle-shaped and occupy the middle third of the fibers (pl. 3, *A*). The remaining two-thirds of the fibers are composed almost entirely of cell-wall substance devoid of pits. Consequently, in a given cross section the lumina may vary from minute to large, since the fibers overlap.

The simple pits, of the flattened funnel-shaped type, radiate out from the lumina in all directions. Hence by means of the pits the fibers communicate with one another, with the epidermis, and with the mesophyll.

The cytoplasm of the fibers often stands out clearly and appears devoid of vacuoles. At times the cytoplasm becomes amber colored, owing to tanniferous infiltrations. The nuclei are large and are generally pressed against the cell walls.

THE STOMATA

Stomata occur along the lateral sides of all spruce needles with the exception of the species in the section *omorika*. In this section the needles are flattened, that is, compressed dorsiventrally, and the stomata are generally found only on the morphological upper side. An exception is *Picea sitchensis* (Bongard) Carrière, which though assigned to this section frequently has stomata on all sides of the needles.

The stomata are arranged in parallel rows extending in the direction of the needles. The number of rows on a given side of a needle is fairly constant in the same species but often varies considerably among different species. For example, in *Picea spinulosa*, 2 rows usually occur, while in *P. polita* Carrière up to 10 have been observed.

The stomata are typically xerophytic, that is, below the surface of the epidermis. In surface view the outer stomatal cavity is delimited by four specialized epidermal border cells. The two lateral border cells are kidney-shaped and extend in the direction of the needle. At either end and separating the lateral cells is a square or trapezoidal end border cell. The arrangement of these border cells imparts a broadly oval or barrel-shaped outline to the stomatal cavity. The outer walls of the border cells—those toward the cavity—appear to be excessively thickened, though actually they are uniformly thickened. The apparent greater thickness is caused by the upward thrust of the accessory cells, which will be discussed in the following paragraphs.

In thick plane sections and by proper downward focusing of the microscope two or four accessory cells may be seen. When two such cells are present, they extend from beneath the center of one end border cell to a similar position below the other, following the contours of the lateral border cells. When four accessory cells are present, they conform in shape to the border cells. The accessory cells are generally broader than the border cells.

Again, by downward focusing in these sections, two kidney-shaped guard cells may be discerned. They also extend in the direction of the needle and are oriented with their concave surfaces toward each other. They are approximately three-fourths as long as the accessory cells.

In cross section the lateral border cells and the accessory cells are roughly triangular in shape. The inclined or lower walls of the border cells slope downward and away from the outer stomatal chamber. The inclined walls of the accessory cells slope in the same direction, but in this case they represent the upper walls. Hence, the inclined walls of the border and accessory cells (in pl. 4, *A*, the cells on either side of the outer stomatal cavity) are joined.

The walls of the accessory cells are unequally thickened. The upper or inclined walls and the outer walls are greatly thickened, their thickest portion being in the region of their junction. From this region the walls gradually thin until they are almost as thin as the lower membranous walls, which can be distinguished only with the aid of an oil-immersion lens.

The length of the inclined walls of the accessory cells is greater than that of the border cells. Hence the inclined walls of the accessory cells project beyond the border cells into the outer stomatal cavity, frequently to the outer surface of the epidermis. It is this projection of the inclined walls of the accessory cells that causes the outer walls of the border cells to appear thickened in plane section.

The structure of the guard cells in cross section is unique. To appreciate it fully, the guard cells should be observed when they are fully expanded and at high magnifications (pl. 4, *A*). In this condition the cells are somewhat teardrop-shaped and are oriented with their rounded or blunt ends toward the stoma. The cells are in contact with the accessory and adjacent cells only along the inner half of the upper wall (that toward the epidermis) and at the extreme inner end of the lower wall. The latter wall may be described as hinged to the hypodermis.

The upper and lower walls curve toward each other at both ends and are connected at the stomatal opening by means of a flexible membranous wall. The membranous wall can be best observed with the aid of an oil-immersion objective.

In 1898 Schwendener (*13*) observed a pitlike structure near the extreme inner end of the upper walls of the guard cells. This is actually a second membranous wall, as is shown by the fact that when the guard cells contract the membrane folds so that the upper wall comes in contact with the lower. So far as can be ascertained, pit membranes do not behave in this manner. When the guard cells are not expanded, the membranous walls adjacent to the stoma appear as dark lines separating the upper and lower walls. In this condition the guard cells assume an elliptical shape in cross section.

The cross-sectional shape of the guard cells near their ends, where they have not separated, is considerably different from that near the center of the cells. Near their ends the cells are right-triangular with the lower walls paralleling the epidermis; the upright or contact walls are at right angles to the lower walls, and the inclined walls slope downward and away from the outer stomatal cavity (pl. 4, *B*). The walls are uniformly thickened, and the membranous walls described previously are not in evidence. However, near the center of the two contact walls there is a thickening of the middle lamella, which evidently corresponds with the outer membranous walls.

THE RESIN CANALS

Two longitudinal series of resin canals occur in the leaves of all species of *Picea*. They are found in the mesophyll adjacent to the dermal tissue and in most species are immediately below the lateral angles of the needles. In the section *omorika* the canals are located on the lower side of the needles at various positions between the lateral and lower angles. The position of the canals is constant in the different species and often serves as a diagnostic character.

The resin canals rarely extend the full length of the needles. They usually occur as longitudinal, fusiform cysts separated by mesophyll tissue. The canals range from 1 to several millimeters long, averaging approximately 2 mm. In some instances a canal on one side of the needle may be continuous for several millimeters, while the canal on the opposite side is broken into two or three widely separated short cysts.

Thus, in a given cross section, no, one, or two resin canals may be present. This feature, which will be discussed in a later paragraph, is important from the standpoint of identification.

Except in two species, the resin canals, as seen in cross section, normally always border upon the epidermis (pl. 1, *A*). The two exceptions are *Picea polita* and *P. sitchensis*. The resin canals in these two species touch upon the hypodermis. The cross sections show further that the diameters of resin canals vary considerably with different species. For example, in *P. sitchensis* the canals measured up to 440 microns, while in *P. likiangensis* Pritzel the largest was only 70 microns.

The resin canals are delimited by two generally uniseriate sheaths of cells. The outer or strengthening sheath is composed of short, thick-walled, fiberlike cells. The cells in cross section are flatly oval and are laterally in contact with one another. The cells are smaller than those of the hypodermis and their walls frequently display a laminated structure.

The strengthening cells are approximately one-third to one-half as long as the hypodermal fibers and have tapering or sharply inclined end walls. The cells with tapering ends overlap. This overlapping causes the sheath to appear biseriate in cross section. The cells with sharply inclined end walls are oriented in vertical series and their end walls abut squarely upon each other. Funnel-shaped simple pits with vertical, elliptical, inner apertures radiate from the lumina in all directions.

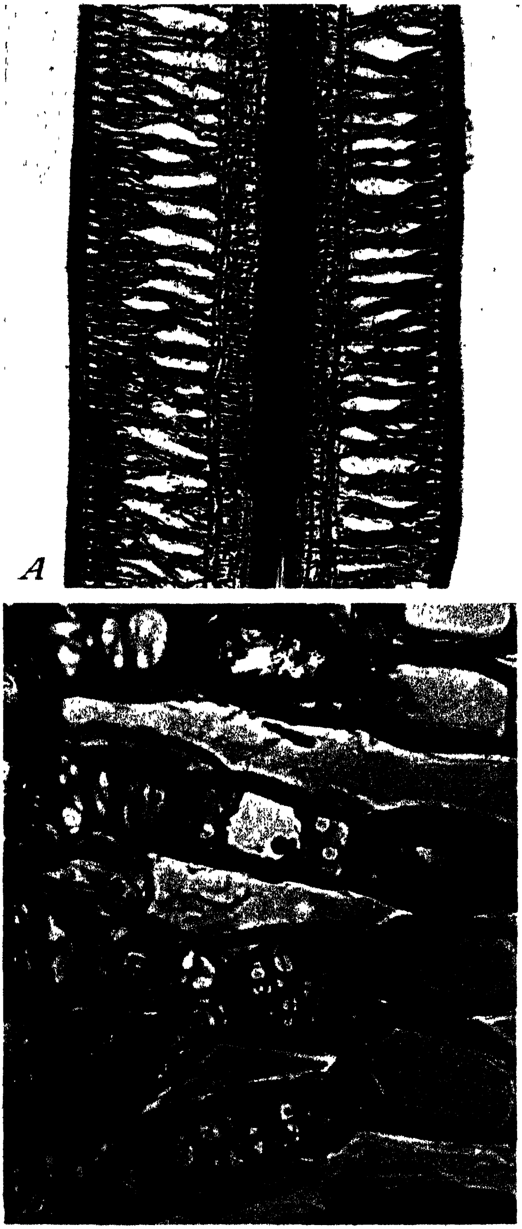
The inner or epithelial sheath is composed of short, thin-walled parenchymatous cells, which are approximately one-half as long as the strengthening cells and extend in the direction of the canals. The cells are somewhat hourglass-shaped and have sloping end walls. The end, lateral, and outer walls are thicker than the inner walls, which often bulge into the canals forming tylosoids. The walls contain numerous round, simple pits which often appear bordered. The bordered appearance of the pits occurs when the inner aperture is larger than the outer. Pits are not as numerous in the inner walls as in the other walls. In cross section the cells are thinly rectangular and wider than the strengthening cells to which they closely adhere.

The cells contain abundant whitish, translucent protoplasm with lenticular nuclei. According to Hannig (*8*) no resinous contents could be found in these cells. He reports that Schwaback, however, using copper acetate, detected fine particles of resin abundant everywhere in the epithelial cells.

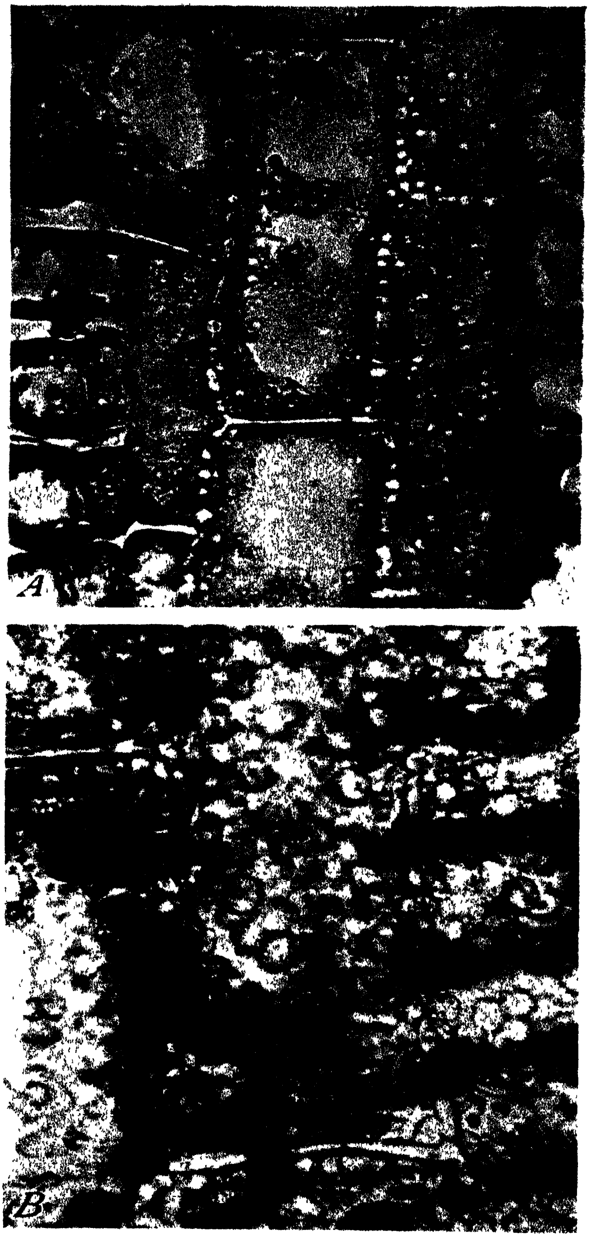
THE MESOPHYLL

Within the hypodermis and extending to the endodermis is the mesophyll, which forms the bulk of the needle. The cells of the mesophyll are large, as compared with those of the other tissues, and contain the chloroplasts.

As seen in cross section, the mesophyll is composed of irregularly arranged, thin-walled cells (pl. 1, *A*). In the majority of the species, the mesophyll is not differentiated into spongy and palisade cells. Palisade cells were observed in *Picea breweriana*, *P. spinulosa*, *P. smithiana* Boiss. (syn. *P. morinda* Link), and *P. brachytyla* Pritzel. These cells, which are roughly rectangular in shape, are generally



A, Longitudinal section through the central portion of a needle of *Picea omorika* (Pancic.) Purkyne illustrating the arrangement of the mesophyll in layers separated by air spaces, × 75; *B*, longitudinal section of *P. sitchensis* near the endodermis showing the simple pits of the mesophyll cells, × 150.



A, Longitudinal section through the endodermis of *Picea breweriana*. Notice that the lateral walls have numerous simple pits in them, while the end or upper and lower walls are devoid of pits. × 400. *B*, Starch grains in the endodermal cells of *P. breweriana*. × 800.

arranged in one or two vertical layers along the sides of the needle adjacent to the hypodermis. The palisade cells are generally more abundant on the morphological lower side of the needle. This is the upper side when attached to the tree, owing to the twisting of the sterigmata. The spongy mesophyll cells are usually isodiametric, as in *P. pungens* Engelm., but they may be oblong or irregular in shape, as in *P. maximowiczii* Regel and *P. asperata*.

The cell walls of both the palisade and spongy mesophyll are slightly but uniformly thickened and, according to Feustel (*5*), strongly lignified. The walls in most species are smooth or slightly wavy but may be strongly infolded or corrugated as in *Picea breweriana*. The adjoining cells are in close contact with one another and usually without interstitial spaces. Interstitial spaces were observed only in *P. wilsonii* Masters, *P. orientalis* Carrière, *P. polita*, *P. sitchensis*, and *P. breweriana*.

The appearance of the longitudinal section is considerably different from that of the cross section. In the former the cells are flatly oblong, sometimes doubly concaved, and are arranged in uniseriate layers separated by air spaces varying in width (pl. 5, *A*).

Toward the hypodermis the layers become two or more cells thick and unite. The mesophyll is thus continuous along the hypodermis for a depth of two or three cells. This continuity of the mesophyll does not occur at the endodermis where the layers maintain their individuality.

Longitudinal sections show that the mesophyll cells are abundantly pitted (pl. 5, *B*). The pits, which are of the simple type, occur in the lateral walls of the cells and rarely in the upper and lower walls except in the region of the hypodermis where the mesophyll layers meet. The pits are best observed in sections tangent to the pit fields, because the plastids that adhere closely to the cell walls obstruct the view of these structures in other sections. Besides the usual inclusions, the cells often contain rather large rhombohedral crystals. These crystals when viewed under polarized light frequently display a variety of colors, principally white, red, and blue.

THE ENDODERMIS

In the central portion of a needle cross section there is a uniseriate, chainlike ring of cells, which is called the endodermis (pl. 1, *B*). Actually, the endodermis is a cylindrical sheath of cells that extends nearly the full length of the needle and encloses the transfusion tissue.

The endodermis in cross section does not show any striking structural characters. However, there are certain features such as the uniformity of cell size and the thickness of the walls that are of distinct diagnostic value for the identification of the various species. For example, in *Picea breweriana*, the walls of the endodermal cells are uniformly thickened, but the cells are unequal in size; in *P. abies* Karst. (syn. *P. excelsa* Link), the outer walls are thicker than the inner, and the cells are equal in size; in *P. polita* the walls are uniformly thickened, and the cells are equal in size; and in *P. spinulosa* the cells are unequal in size and irregularly shaped, and the walls are uniformly thickened. Starch grains have been found in the endodermal cells (pl. 6, *B*).

Longitudinal sections show that the endodermal cells are usually rectangular in shape and arranged vertically in horizontal rows. The vertical, i. e., the tangential and radial, walls contain numerous well-defined, simple pits. No pits are evident in the transverse, or end, walls (pl. 6, *A*). The pits in the material studied always led to adjacent cells and none were observed leading to air spaces. According to Soar (*14*) the walls of the endodermal cells are lignified. In addition, the transverse, or end, walls are heavily suberized. This suberization and lignification plays a definite role in the function of the endodermis, as will be discussed in more detail later.

THE VASCULAR TISSUE

Within the endodermis is located the transfusion tissue. This tissue can be conveniently divided into the fibrovascular bundle and transfusion cells (pl. 1, *B*).

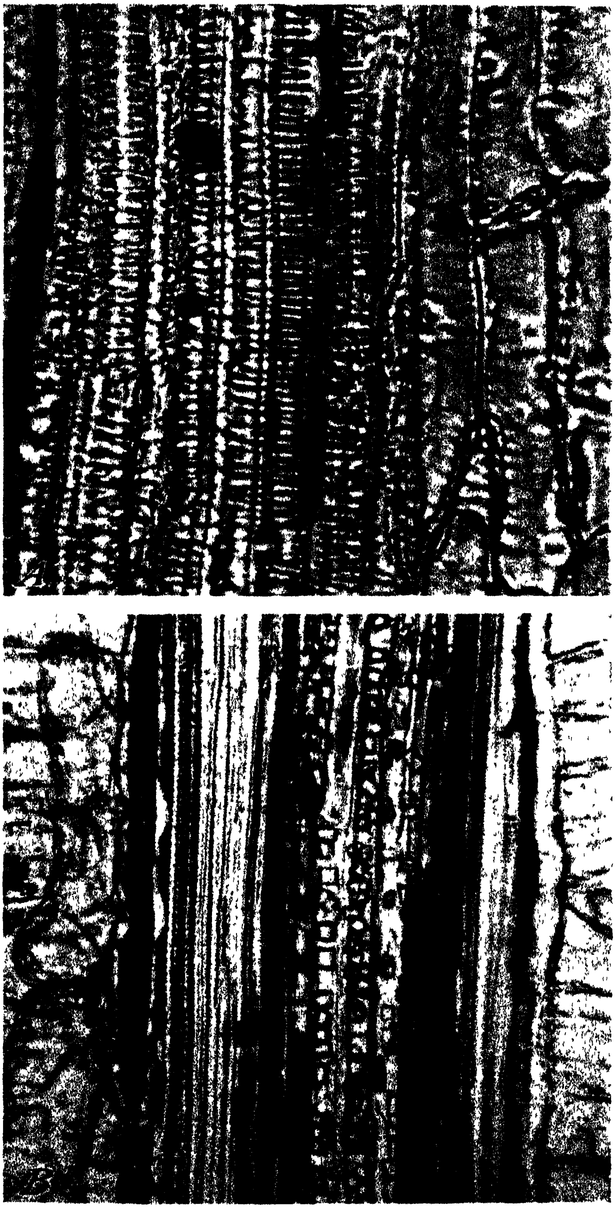
Fibrovascular Bundle

Although the spruces are generally credited with having but one fibrovascular bundle in their leaves, there is evidence that at an earlier evolutionary period there might have been two bundles. Lascassagne (*11*) states that in the cortex (sterigmata) the bundles of the leaf trace are distinct but in the leaves they are fused. In all species of spruce the bundles are distinctly divided, each part having an equal amount of xylem and phloem. Further, the phloem in many of the species is separated into two divergent wings.

The xylem forms the morphological upper side of the bundle and is composed of primary and secondary tracheids, parenchyma, and fibers. The primary tracheids are located at the lower margin of the xylem and comprise the first two or three rows. These cells have large bordered pits and distinct annular thickenings which may be closely or loosely arranged (pl. 7, *A*). The cells are short, thin-walled, and have distinct end walls. Generally each end wall contains a simple, large, bordered pit. The secondary tracheids are longer than the primary, thicker walled, and have long, tapering, overlapping ends. The cells are devoid of annular or spiral thickenings and have numerous bordered pits.

Xylem or wood parenchyma was found in all the species studied. It is generally arranged in uniseriate, vertical, raylike sheaths, which separate the bundles into equal parts (pl. 1, *A*). The cells, which are also arranged vertically, are short, oblong, and thick-walled and have numerous simple pits in their walls. Some observers who have studied only the cross section of the needles have reported medullary rays in the xylem, especially in those species with large bundles, such as *Picea breweriana* and *P. polita*. Study of the longitudinal sections shows that these rays are parenchyma sheaths similar to those described. The cells of the parenchyma sheaths frequently contain crystals (pl. 7, *B*).

The phloem also can be divided into primary and secondary. The primary phloem comprises two or three rows of cells along the lower margin of the bundle. The cells are compressed or crushed so that their lumina are not distinguishable. This crushing of the primary phloem seems to indicate that secondary growth takes place in the bundles. The secondary phloem cells are not as long as the tracheids and have well-defined lumina. The cells are thin-walled, usually blunt-ended, and have two to four sieve plates. They also have definite nuclei and cytoplasm. Companion cells were not observed.



A, Longitudinal section through bundle of *Picea breweriana* showing primary xylem with annular thickenings and secondary xylem with large border pits, × 800; *B*, longitudinal section through the center of the bundle of *P. breweriana* depicting the crystals that are frequently found in the phloem parenchyma cells, × 400.

TRANSFUSION CELLS

Within the endodermis and surrounding the fibrovascular bundle are the transfusion cells. These cells are of three types—parenchymatous, tracheal, and fibrous. The parenchymatous cells are nearly isodiametric, comparatively large, thin-walled, and simply pitted, and have conspicuous nuclei. The pits of these cells lead to similar cells, to cells of the phloem, or to endodermal cells. In the material studied, pits leading to the tracheal elements were uncommon. These parenchyma cells also contain oil globules, starch granules, and crystals.

The tracheal elements have thicker walls than the parenchymatous cells, and according to some authors, the walls are lignified. The cells are devoid of cell contents, are more or less rectangular, and have conspicuous bordered pits (pl. 3, *B*), which lead to other tracheal elements and to the endodermal cells.

The fibrous elements, or strengthening cells, are similar in all respects to the libriform fibers of the hypodermis. The fibers are long to very long with tapering end walls. The walls are very thick, lignified, and often display a laminated structure. The pits are simple and of the flattened, funnel-shaped type. These fibers are present in nearly all species above and adjacent to the phloem (pl. 1, *B*). In *Picea breweriana* and *P. brachytyla* the fibers are also found below the xylem. The abundance of these fibers can often be of use in the identification of the species. For example, in *Picea smithiana* they are usually 10 to 30 in number, while in *P. spinulosa* they are generally absent.

FUNCTIONS OF THE TISSUES

The functions of most of the leaf tissues are fairly clearly understood. It is generally recognized that the epidermis serves as protection; the stomata for regulating transpiration; the mesophyll for the elaboration of food products; and the fibrovascular bundles for the conduction of water and the translocation of the synthesized foods. On the other hand, the functions of the hypodermis, endodermis, and transfusion cells have not been clearly defined.

As already described, the hypodermis is composed of long to very long libriform fibers, which have very thick walls and long, tapering, overlapping ends. These cells are comparable to those found in wood, where their chief function is for strength and support. It is logical to assume that in the spruce needle also the primary function of these cells is for strength and support. Their secondary functions are for protection and, by virtue of their living cell contents, as intermediate agents for supplying the epidermal cells with the necessary nutrition materials. The fibrous elements adjacent to the resin canals serve in the same capacity, but those adjacent to the bundles must function purely for mechanical support, since living cell contents are not present in them.

Soar (*14*) intimates that the endodermis functions as a regulator for the passage of water to the mesophyll, since the radial and tangential walls are lignified and the transverse walls are suberized. This deduction is probably true, but the present study seems to indicate that water regulation may be a secondary function.

Leaves of hardwood trees have capillary veins which reach out into the mesophyll to collect and distribute evenly water and elaborated foods. Frequently the capillary veins are reduced to a single tracheid, which is surrounded by specialized parenchyma or mesophyll cells. These specialized parenchyma or mesophyll cells function as intermediate agents for the collection and distribution of foods and water. In conifer leaves capillary veins are absent. Consequently, some tissue or group of cells must take over their function. The structure of the endodermis strongly suggests that one of its primary functions is the even distribution and collection of water and foods. As already mentioned, the fibrovascular bundles in the spruces are completely cut off from the mesophyll by the endodermis. Further, the endodermis is composed of specialized parenchyma cells, which are arranged in more or less horizontal rows. The cells in a given row are independent of the cells in the rows immediately above or below, because of the heavily suberized end walls devoid of pits. These facts, coupled with the layering of the mesophyll, indicate that a single row of endodermal cells insures the even distribution and collection of water and foods from the adjoining mesophyll layer.

The structure of the transfusion cells also indicates the passage of water from the fibrovascular bundle to the endodermis, and the return to the bundle of elaborated foods. The tracheal cells are lignified and have bordered pits—features typical of water-conducting elements. The tracheids communicate by means of their bordered pits with similar cells, with the endodermis,with the xylem of the bundle, and, rarely, with other cells. Hence, a tracheal strand figuratively forms a direct passage for water from the bundle to the endodermis. Similarly the parenchyma cells, which are characterized by living cell contents, unlignified walls, and simple pits, form the passageway for elaborated foods to get to the phloem from the endodermis. This hypothesis is substantiated by the fact that starch grains, crystals, and oil globules are common inclusions of the parenchyma cells, while they are entirely lacking in the tracheal cells.

The continuity of pitted cells from the fibrovascular bundle to the epidermis should be pointed out here. This continuity means that water and elaborated foods in solution pass from cell to cell by osmosis through the pit membranes and not through the semilignified cell walls as is commonly thought.

SUMMARY

The present study of spruce needles has revealed several interesting anatomical features, some of which have supplied the answers to a few much mooted questions. These features are as follows:

The epidermal cells are equipped with teeth which project into the cuticle. These teeth help to anchor the cuticle firmly into position.

The epidermal cells are locked together by a modified dovetailed joint.

The variation in the size of the lumina of the libriform hypodermal fibers is not of diagnostic value, since the fibers overlap.

The structure of the guard cells is unlike any heretofore described or portrayed. In particular, the thickened upper and lower walls of the guard cells in the region of the stoma are separated by two extremely thin, flexible membranes. When the stomata are opened, the membranes collapse and the two guard-cell walls come in contact.

All spruce needles have two longitudinal, parallel series of resin ducts or cysts. These ducts or cysts are usually located in the lateral angles of the needles adjacent to the dermal tissue. In a given cross section no, one, or two ducts or cysts may be evident. Consequently, the number of ducts or cysts per section is not of diagnostic value. The diameters of these structures, however, are constant within certain limits and can be used in identification.

The mesophyll cells are arranged in uniseriate layers, separated by air spaces. The cells are equipped with rather large simple pits.

The endodermis is a uniseriate, cylindrical sheath of cells which separates the transfusion tissue from the mesophyll. By virtue of its pitting and the chemical nature of its cell walls the endodermis serves as a distributing and collecting agent for water and elaborated foods.

The transfusion cells are chiefly tracheal and parenchymatous. The two types of cells serve respectively in the conduction of water and elaborated foods.

The number of strengthening, or fibrous, cells adjacent to the bundles is of diagnostic value.

The spruces have two fibrovascular bundles in their petioles, which become fused into one in the needle. The bundle is separated vertically into two equal parts by a raylike sheath of parenchymatous cells.

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INFLUENCE OF MOISTURE AND LOW TEMPERATURE ON THE GERMINATION OF HOP SEEDS[1]

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INTRODUCTION

Though hop plants (*Humulus lupulus* L.) are propagated commercially by the use of cuttings from underground stems, breeding work requires the sexual propagation of the species. The writer became interested in the possibilities of low temperatures in stimulating the germination of hop seeds because of the relation of this problem to varietal improvement. Investigations in progress included the growing of seedlings for observation, selection, and determination of the results of controlled pollination with numerous individual plants. Germination of most seed lots was extremely low, ranging from 0 to 10 percent even after they had remained under apparently favorable germination conditions for several weeks. Seedlings developed from very few of the numerous seeds sown, and the number of plants from which to make selections was therefore small and the difficulties of studying the effects of controlled pollination were greatly increased. For these reasons experiments were undertaken, the results of which are presented here.

The present work is concerned with the germination aspects of treatments of hop seeds, including moisture and temperature factors. The term "dormancy" is used in a general sense, no attempt having been made to study the nature of the seed in its relation to germination. The investigations were made at Corvallis, Oreg.

REVIEW OF LITERATURE

In recent years much has been written concerning the effects of various treatments on the physiological behavior of seeds and plants. The results of subjecting dormant seeds or seeds in early stages of germination to controlled-moisture and low-temperature conditions have been studied both by those interested in germination and by others concerned principally with the effects on subsequent growth.

Crocker (*2*) [2] and Toole (*9*) presented discussions of the general aspects of seed dormancy and indicated certain factors as influential in hastening afterripening. Pertinent literature was also cited by these writers. Nichols (*6*) studied the influence of low temperatures on seed germination and found growth of certain native species to be hastened by exposure to cold. Mirov (*5*) reported that low-temperature treatments stimulated germination in certain coniferous species.

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[2] Italic numbers in parentheses refer to Literature Cited, p. 380.

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Haut (*4*) found low-temperature storage at several temperatures to stimulate afterripening and promote germination of pear, apple, cherry, and peach seeds.

Raum (*7*), in a discussion of hop-improvement problems, directed attention to the difficulty of obtaining prompt and complete germination of hop seeds. Sprouting was found to be almost complete, however, if seeds were placed in pots and subjected to outdoor conditions during the winter until April. Bressman (*1*) referred to the use of chilling and scarification in stimulating hop seed germination. The treatment suggested was 10 days at freezing temperatures followed by scarification with a coarse emery cloth.

Though other workers in hop breeding have undoubtedly encountered the problem of initial low germination of hop seeds under certain methods of handling, no references to this subject other than those noted have been seen by the writer.

MATERIALS AND METHODS

In preliminary experiments, 1934, seeds of Late Clusters, Early Clusters, Fuggles, and Red Vine, principal hop varieties of the Pacific coast, were placed upon moist blotters in Petri dishes. Conditions for germination were kept favorable with respect to moisture, and temperatures ranged from 15° to 23° C. The dishes were kept in indirect light. A similar series was incubated in blotters in a standard germination chamber at room temperature. No pretreatments of any kind were given, but midway through the period the seeds and substratum became very moldy. The seeds and Petri dishes were then washed in a dilute solution of an organic mercury disinfectant, new blotters were supplied, and incubation was continued as before.

Incidental tests were made also with several periods of refrigeration at different low temperatures. Eight lots of seeds of each of the four varieties were placed in cold storage at 5° and −12° C. for periods of 2, 4, and 6 weeks at the former and 2 weeks at the latter temperature. The seeds were sown in soil at the termination of the periods, and observations were made on germination.

A more extensive experiment was completed in 1936. Lots of seeds of the four varieties previously studied were tested with four incubation periods before cold storage, five periods of refrigeration, and two temperatures. The combinations of these variables in treatment series comprised 40 tests of each variety, as listed in table 2. The tests were made in triplicate, and three check series were also included. Check 1 consisted of seed lots that received no wetting, incubation period, or cold storage but were sown, without any treatment, direct in the soil. Check 2 was not wetted or incubated in the germinator but was stored 2 weeks at −12° C. Check 3 was treated like check 2 except that the cold storage was at 5°.

Dry seeds were counted out in lots of 100 each. They were then placed in folded, moist paper toweling and incubated in a standard germinator for the periods indicated. Care was taken to avoid exclusion of air in folding the toweling. At the end of the incubation period the lots were immediately placed in refrigeration and left for the desired time. They were then removed, and the seeds were planted one-half inch deep in short rows in greenhouse benches. Lots of the —12° C. series were allowed to thaw 24 hours at about 70° F. before seeding.

The term "incubation period," as used herein, refers to the period in the germinator during which no seeds germinated. Germination percentages were noted at intervals of 2 weeks from the date of seeding of each lot, percentages being recorded for 2, 4, 6, and 8 weeks after seeding.

In order to obtain further information on the variability of germination among seed lots from different plants, germination tests were made with seeds of the same crop year from 204 individual plants. All lots were first subjected to 2 weeks' refrigeration at 5° C. One series was then sown in field coldframes without bottom heat with outdoor temperatures ranging from 20° to 80° F. A second series was sown in greenhouse beds, with temperatures during germination ranging from 40° to 90° F. Material planted in coldframes was subject to seasonal rains; that in the greenhouse was artificially watered.

It is a common observation among growers that hop seedlings begin to appear in late January or February under field conditions in western Oregon. These develop from seeded hop cones that fall to the ground during harvest or remain on the vines when the latter are cut down following picking. The seeds evidently undergo a rest period in the field and begin to germinate in late winter. In the fall and winter of 1935–36 seeds were gathered September 25, November 25, and January 25 from the same individual plants in the field. The plants represented unnamed seedlings varying widely in morphological characters. After the collection of the last lot the samples of the three collections were seeded in greenhouse benches, and observations were made on germination. This test was not replicated.

In the experiments outlined, care was taken to include only sound seeds, since many apparent "seeds" are only hulls. Seeds of varieties and of individual plants varied in size to some extent. In threshing the seeds from the strobiles by hand the persistent perianth was not removed. No seeds germinated in the incubation period and none were observed to be markedly swelled by this treatment. Unless otherwise noted, 100 seeds were included in each lot.

The soil used in the cultures, both field and greenhouse, was a medium sandy loam of the Chehalis series, and no commercial or other fertilizer was applied. The soil was kept reasonably moist. Seeds were considered to be germinated when the seedlings were beginning the third leaf. No damping-off or other seedling disease was noted in any of the trials. During the period of germination greenhouse temperatures varied between 45° and 90° F., the most frequent range being from 60° to 80° F. The type of refrigeration used was that provided by a small ammonia plant with temperatures kept reasonably constant. No variations of more than 3° C. above or below the temperatures indicated were observed.

RESULTS

PRELIMINARY GERMINATION TESTS

Data for the preliminary tests of germination in blotters and Petri dishes are given in table 1. The figures represent additional seeds that germinated at each successive date. Since there were but 50 seeds in each lot, percentage may be derived by multiplying the figures by 2 in each instance. Though the Petri dishes were kept in daylight and the blotters in a standard germinator, it is obvious that even after long periods seeds may fail to sprout. Very few seeds softened during the period.

TABLE 1.—*Germination of hop seeds of several varieties in Petri dishes and germination blotters* [1]

Variety	Additional seeds germinated after indicated incubation period (days) in—																			
	Blotters										Petri dishes									
	15	20	25	30	35	45	50	60	70	75	15	20	25	30	35	45	50	60	70	75
	No	*No.*	*No*	*No.*	*No*	*No.*	*No.*	*No.*	*No.*	*No*	*No.*	*No*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No*
Red Vine	4	1	0	0	0	0	1	0	0	0	1	2	1	0	0	0	0	0	0	0
	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fuggles	1	0	0	0	0	3	1	0	0	1	1	0	0	1	0	0	0	1	1	1
	0	0	0	1	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Late Clusters	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
	0	0	0	0	1	0	1	0	0	0	0	0	1	0	1	0	0	1	0	1
Early clusters	0	0	0	1	0	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

[1] 50 seeds were used in each test.

Additional preliminary data, derived from subjecting to low temperatures seeds from the same lots as those used in the blotter and Petri-dish tests, indicated that germination was modified favorably thereby. Though the trials were cursory, relative effects of refrigeration periods showed 6-, 4-, and 2-week periods at 5° C., 2 weeks at —12°, and the check to rank in the order named in germination percentage.

MOISTURE AND TEMPERATURE RELATIONS

Table 2 contains data secured from a more extensive study of the interrelation of varieties, periods of incubation before refrigeration, refrigeration periods, and refrigeration temperatures with germination. Though little attention is given to the germination data for other than the final germination date, the values for the earlier dates may be of interest and are therefore included. Each germination value in table 2 represents an average of three replications. Check 1 included seed lots without treatment; checks 2 and 3 were held for 2 weeks at —12° and 5° C., respectively.

In table 3 the averages of each treatment for the last date of observation have been recorded to facilitate comparisons.

In general, variations between replicates were large. Throughout the 40 experimental series, percentage germinations ranged from very low to fairly high values, as the data indicate. Inclusion of all series in a determination of a generalized error, such as the analysis of variance, was therefore inadvisable. It was possible, however, to analyze series 23 to 25, 28 to 30, 33 to 35, and 38 to 40 by such a method (*3*) without serious infringement on data limitations. The results so analyzed are for the eighth-week observations only.

Table 2.—*Effect of various periods of incubation and refrigeration (at —12° and 5° C.) on germination of 4 varieties of hops 2, 4, 6, and 8 weeks after seeding*

Series No.	Incubation in germinator	Refrigeration		Germination[1] in indicated number of weeks after seeding—															
		Duration	Temperature	Early Clusters				Late Clusters				Fuggles				Red Vine			
				2	4	6	8	2	4	6	8	2	4	6	8	2	4	6	8
	Days	*Weeks*	°*C.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct*	*Pct*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
1	2	1	−12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.33	0.33	0.67	0.00	0.00	0.00	0.33
2	2	2	−12	.00	.00	.00	.33	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
3	2	3	−12	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.33	.33	.00	.33	.67	.67
4	2	4	−12	.00	.00	.67	.67	.00	1.67	2.33	2.33	.00	.67	1.00	1.00	.33	2.00	2.00	2.00
5	2	5	−12	.00	.00	.00	.67	.00	1.67	2.67	3.33	.33	.67	.67	1.33	.33	1.67	2.33	3.33
6	5	1	−12	.00	.00	.00	.33	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
7	5	2	−12	.00	.00	.00	.00	.00	.00	.33	.33	.00	.00	.00	.33	.00	.67	1.00	1.33
8	5	3	−12	.00	.00	.00	.00	.00	.00	.00	.33	.00	.00	.00	.00	.00	.33	1.33	1.33
9	5	4	−12	.00	.00	.33	1.33	.00	.00	.00	.33	.00	.33	.33	1.00	.00	.00	.00	2.67
10	5	5	−12	.00	.33	.33	2.00	.00	4.00	5.33	5.67	.33	.33	.67	.67	.00	3.33	6.00	9.67
11	10	1	−12	.00	.00	.00	.00	.33	.33	.33	.67	.00	.00	.00	.33	.33	.33	.67	1.00
12	10	2	−12	.33	.33	.67	.67	.00	.00	.33	.67	.00	.00	.00	.00	1.00	4.33	6.00	7.00
13	10	3	−12	.00	.33	.33	.33	.33	.33	.33	.33	.00	.00	.00	.00	.00	.33	1.33	1.33
14	10	4	−12	.00	.00	.00	.33	.00	.33	.67	.67	.00	.33	.33	1.00	.33	.33	.33	1.67
15	10	5	−12	2.00	9.33	13.00	13.00	3.00	14.67	16.67	16.67	1.00	16.33	20.33	20.33	3.00	27.33	34.33	34.33
16	15	1	−12	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.67	1.00
17	15	2	−12	.00	.33	1.67	1.67	.00	.00	.00	.00	.00	.67	.67	.67	.33	1.00	1.00	1.00
18	15	3	−12	.33	14.33	24.00	24.33	.00	4.00	6.67	7.67	.00	7.67	19.67	24.33	.00	22.67	38.00	42.67
19	15	4	−12	.33	3.33	8.67	8.67	2.00	17.67	25.67	25.67	5.33	19.00	23.00	23.00	1.33	15.67	23.67	23.67
20	15	5	−12	2.67	15.00	20.33	20.33	.33	8.33	14.00	14.00	.33	4.33	14.33	14.33	4.33	19.67	23.00	23.00
21	2	1	5	.00	.00	.00	.00	2.00	2.67	3.00	3.67	.00	.00	.00	.33	.00	.33	1.00	2.00
22	2	2	5	4.67	5.00	5.00	5.00	13.33	15.33	16.33	16.33	.00	.00	.67	1.00	6.00	6.67	7.67	9.00
23	2	3	5	6.00	9.67	10.67	10.67	30.33	39.33	41.33	41.67	5.00	12.00	13.67	13.67	19.33	26.67	27.33	27.33
24	2	4	5	40.00	48.67	49.67	49.67	44.00	52.33	52.67	52.67	35.67	40.67	42.67	42.67	51.00	61.00	61.33	61.33
25	2	5	5	32.00	63.67	64.00	64.67	24.67	45.33	46.33	47.00	29.00	70.33	72.00	72.67	33.33	66.67	67.33	68.00
26	5	1	5	.67	.67	.67	1.33	4.00	5.33	5.33	5.33	.00	.00	.33	1.33	2.67	3.67	4.00	5.00
27	5	2	5	14.67	16.67	17.00	17.00	7.33	9.00	9.33	9.33	.00	3.33	4.00	4.33	11.33	12.33	12.67	13.00
28	5	3	5	13.33	18.67	19.67	19.67	3.67	10.00	10.67	10.67	8.00	12.33	14.33	15.00	1.00	6.00	6.67	7.67
29	5	4	5	.33	6.67	14.67	18.67	.67	8.33	11.67	13.00	.67	7.00	14.00	17.33	.67	6.33	12.67	13.33
30	5	5	5	58.00	77.00	78.33	81.33	60.00	67.00	67.33	69.00	42.33	80.67	81.33	81.67	58.00	68.33	68.33	68.33
31	10	1	5	.00	.00	.00	.33	1.67	1.67	1.67	2.00	.00	.00	.33	.33	1.33	1.33	1.33	2.33
32	10	2	5	13.00	17.67	19.67	20.00	16.33	22.33	23.67	23.67	1.00	5.33	8.33	9.00	14.67	22.67	23.00	23.33
33	10	3	5	21.67	30.00	32.00	32.00	26.67	36.67	37.67	37.67	29.00	38.00	39.67	39.67	21.33	31.67	34.33	34.33
34	10	4	5	9.33	25.33	26.00	26.33	25.00	45.33	46.00	46.33	18.00	32.67	34.00	34.00	25.67	48.33	49.33	49.67
35	10	5	5	23.33	58.67	61.33	61.33	41.33	57.00	57.33	57.33	46.67	62.00	63.00	63.00	30.00	48.67	51.00	51.00

[1] Each value is an average of 3 replications.

TABLE 2.—*Effect of various periods of incubation and refrigeration (at —12° and 5° C.) on germination of 4 varieties of hops 2, 4, 6, and 8 weeks after seeding*—Continued

Series No.	Incubation in germinator	Refrigeration		Germination in indicated number of weeks after seeding—															
		Duration	Temperature	Early Clusters				Late Clusters				Fuggles				Red Vine			
				2	4	6	8	2	4	6	8	2	4	6	8	2	4	6	8
	Days	*Weeks*	° *C.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct*	*Pct*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct*	*Pct*	*Pct.*	*Pct*	*Pct.*
36	15	1	5	0.00	0.33	0.67	0.67	0.67	1.33	1.67	1.67	0.00	0.33	0.33	0.33	1.67	1.67	2.33	2.33
37	15	2	5	5.33	7.00	7.33	7.33	17.00	18.33	19.00	19.00	1.00	3.00	3.00	3.00	13.33	14.67	14.67	14.67
38	15	3	5	.00	22.00	32.00	32.33	1.33	40.33	44.67	45.67	.33	35.00	33.67	39.67	2.67	30.67	35.33	36.67
39	15	4	5	19.33	41.33	41.33	41.33	24.67	48.00	48.33	48.33	33.67	55.67	58.00	58.00	22.67	45.33	46.33	46.33
40	15	5	5	24.67	46.00	48.00	48.00	21.67	46.67	47.00	47.00	12.67	36.00	37.67	37.67	27.67	44.33	44.67	44.67
Check:																			
1[1]				.00	2.00	2.00	2.33	.00	2.33	6.33	7.33	.00	.00	.00	.33	.33	.67	5.67	6.33
2[3]				.00	.67	1.67	1.67	.67	1.67	3.33	3.33	.00	.67	1.33	1.67	14.33	16.00	17.00	17.00
3[4]				.33	.67	1.33	1.67	.00	.67	1.33	2.00	.00	.33	.67	1.33	1.33	2.67	3.00	4.00

[1] Seeds received no treatment. [3] Seeds were stored 2 weeks at —12° C. without other treatment [4] Seeds were stored 2 weeks at 5° C. without other treatment.

TABLE 3.—*Mean germination percentages of three tests for varieties, for incubation and refrigeration periods, and for temperatures of refrigeration*

Variety	Incubation in germinator	Germination after indicated refrigeration period (weeks) at specified temperature									
		1		2		3		4		5	
		−12° C.	5° C.	−12° C.	5° C.	−12° C	5° C.	−12° C	5° C.	−12° C.	5° C.
	Days	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
Early Clusters	2	0.0	0.0	0.3	5.0	0.0	10.7	0.7	49.7	0.7	64.7
Late Clusters	2	.0	3.7	.0	16.3	.0	41.7	2.3	52.7	3.3	47.0
Fuggles	2	.7	.3	.0	1.0	.3	13.7	1.0	42.7	1.3	72.7
Red Vine	2	.3	2.0	.0	9.0	.7	27.3	2.0	61.3	3.3	68.0
Average		.25	1.50	.08	7.80	.25	23.35	1.50	51.60	2.15	63.10
Early Clusters	5	.3	1.3	.0	17.0	.0	19.7	1.3	18.7	2.0	81.3
Late Clusters	5	.0	5.3	.3	9.3	.3	10.7	.3	13.0	5.7	69.0
Fuggles	5	.0	1.3	.3	4.3	.0	15.0	1.0	17.3	.7	81.7
Red Vine	5	.0	5.0	1.3	13.0	1.3	7.7	2.7	13.3	9.7	68.3
Average		.08	3.23	.48	10.90	.40	13.28	1.33	15.58	4.53	75.08
Early Clusters	10	.0	.3	.7	20.0	.3	32.0	.3	26.3	13.0	61.3
Late Clusters	10	.7	2.0	.7	23.7	.3	37.7	.7	46.3	16.7	57.3
Fuggles	10	.3	.3	.0	9.0	.0	39.7	1.0	34.0	20.3	63.0
Red Vine	10	1.0	2.3	7.0	23.3	1.3	34.3	1.7	49.7	34.3	51.0
Average		.50	1.23	2.10	19.00	.48	35.93	.93	39.08	21.08	58.15
Early Clusters	15	0	.7	1.7	7.3	24.3	32.3	8.7	41.3	20.3	48.0
Late Clusters	15	.0	1.7	.0	19.0	7.7	45.7	25.7	48.3	14.0	47.0
Fuggles	15	0	.3	.7	3.0	24.3	39.7	23.0	56.0	14.3	37.7
Red Vine	15	1.0	2.3	1.0	14.7	42.7	36.7	23.7	46.3	23.0	44.7
Average		.25	1.25	.85	11.00	24.75	38.60	20.28	47.98	17.90	44.35

The results of the use of the analysis of variance are shown in table 4. The data indicate significant differences to exist among refrigeration and incubation periods. The first-order interaction between refrigeration and incubation periods is also significant. Though the comparison of temperatures of refrigeration was necessarily omitted from the analysis, reference to table 2 and comparison of series 1 to 20 with series 21 to 40 make clear at once that a highly significant difference exists. Varietal differences were not significant.

TABLE 4.—*Analysis of variance data on varieties and incubation and refrigeration periods*

Variation due to—	Degrees of freedom	Sums of squares	Mean square	F [1]
Refrigeration periods	2	26,123	13,062	[2] 96.0
Incubation periods	3	2,825	942	[2] 6.9
Varieties	3	141	47	2.9
Refrigeration×incubation	6	17,412	2,902	[2] 21.3
Refrigeration×varieties	6	1,716	286	2.1
Varieties×incubation	9	1,626	181	1.3
Varieties×incubation×refrigeration	18	3,051	170	1.3
Error	96	13,092	136	
Total	143	65,986		

[1] Snedecor (8).

[2] Exceeds 0.01 point.

The standard error of a single analysis is 11.66 percent; the standard error of a mean of three tests, 6.73 percent. Since variety differences were not significant, data for all varieties have been grouped in table 5 for comparison of germination and refrigeration periods. The minimum significant difference for refrigeration general means, based upon the 0.05 point, is 4.75 percent. The 3-, 4-, and 5-week periods, therefore, differ significantly in average germination. For comparing incubation-period means, the least significant difference is 5.50 percent. Accordingly, average germination was significantly lower following 5 days' incubation for all series. It should be borne in mind that these errors apply only to the series included in the analysis of variance.

TABLE 5.—*Mean percentage germination for all varieties for different refrigeration and incubation periods*

Incubation period (days)	Germination after refrigeration for—			Average germination
	3 weeks	4 weeks	5 weeks	
	Percent	*Percent*	*Percent*	*Percent*
2	23.4	51.6	63.1	46.0
5	13.3	15.6	75.1	34.7
10	35.9	39.1	58.2	44.4
15	38.6	48.0	44.4	43.7
Average	27.8	38.6	60.2	42.2

While the check series are not included in the calculation of error, most treatments show unquestionably significant increases in germination when compared with the check lots. The high germination of the Red Vine variety in check 2 is inexplicable.

Within refrigeration and incubation periods he least significant difference, twice the standard error of a difference, is 9.52 percent. On the basis of this error, incubation periods may be compared in relation to the several periods of refrigeration.

Considering lots incubated 2 days in the germinator and refrigerated at 5° C., those left for 4 weeks at low temperatures showed significantly higher germination than those refrigerated for 3 weeks, as an average of all varieties. Lots refrigerated 5 weeks were significantly higher in germination than those remaining at low temperatures for 3 weeks.

The superiority of longer periods of refrigeration following additional days in the germinator was not so marked. Comparing lots incubated in the germinator 10 days before chilling, 5 weeks' refrigeration was better than 4. Among lots incubated in the germinator 15 days before cold storage, refrigeration for 4 weeks was significantly superior to 3 weeks' treatment.

In the lots refrigerated 3 weeks, those incubated 10 and 15 days were significantly higher in germination than those incubated 2 or 5 days. Groups incubated 2 days were also significantly higher than the 5-day incubated groups.

Five-day incubated lots were significantly lower than 2-, 10-, or 15-day lots when refrigerated 4 weeks. The 2-day lot was significantly higher than that incubated 10 days.

When the lots refrigerated 5 weeks after different incubation periods are considered, additional relations are noted. Two-day incubation lots were superior to the 15-day series. Five-day incubation lots were significantly superior to all other groups when refrigerated for 5 weeks. Ten days' incubation was significantly better than 15 days. Fifteen-day incubation groups were significantly lower than others.

The value of *F* also indicated a significant interaction between incubation and refrigeration periods. Examination of the data in table 5 shows that in lots refrigerated 3 weeks, long periods of incubation were followed by increased germination, while with 5 weeks' refrigeration long incubation periods resulted in decreased germination. Using the extremes as an example,

$$(23.4-63.1)-(38.6-44.4)=-33.9$$

and the least significant difference in this comparison is 13.46 percent when based on twice the standard error of the difference. Since the trends of the differences are not closely consistent, it is possible that uncontrolled factors may have influenced the results.

Considering grouped averages of the entire 40 series, certain generalizations may be made. The greatest increase in germination occurred between the second and fourth weeks after seeding. Lots refrigerated at 5° C. showed a much faster germination rate the first 2 weeks and a somewhat more rapid progress the third and fourth weeks than those stored at −12°. It should be noted that some of the seeds sprouted during the fifth week at 5°, before removal from refrigeration. Most seeds germinating in all lots did so by the end of 4 weeks, with only slight increases thereafter.

Successive weeks in cold storage at either temperature resulted in consistent increases in germination, with no tendency toward decrease indicated, except in series remaining 15 days in the germination chamber. Five weeks at −12° C. gave results very similar to those obtained from storage for 2 weeks at 5°. Seed lots stored at −12° showed consistently increased germination as the periods of incubation were prolonged, and no tendency to decrease is indicated.

COMPARISON OF GERMINATION IN GREENHOUSE AND COLDFRAMES

Germination percentages on 40 lots of seed from individual plants seeded in greenhouse beds and field coldframes are listed in table 6. These are a random sample of 204 lots studied and are listed to indicate plant variations and germination rates. These data, though unreplicated, indicate wide variations in both rapidity and amount of germination in lots of seed from different plants. Many of these differences are undoubtedly significant. Since seed was collected only when the cones were well browned and dry, differences in maturity would appear to be of little consequence in explaining variation among plants.

Average germination for 204 lots in the greenhouse at the end of 60 days was 21.17±0.87 percent, while that in coldframes was 18.85 ±0.85 percent. The *P* value for the difference does not quite reach the 0.05 point. The coefficient of correlation between percentages at 60 days, based on 196 plant lots, was 0.19. The *P* value, according to Fisher's *t* test (*3*), exceeds the 0.01 point. The slight average increase in greenhouse-germinated seed may have been due to the higher temperatures existing, better controlled moisture conditions, or other factors. There are instances listed where the field-planted seeds greatly exceeded in germination the same lots seeded in the greenhouse,

TABLE 6.—*Germination percentages of seed lots from individual plants sown in greenhouse or field after 14 days' incubation in moist blotters followed by 14 days' refrigeration at 5° C.*

Plant No.	Germination in indicated number of days after seeding in—					Plant No.	Germination in indicated number of days after seeding in—				
	Greenhouse			Field			Greenhouse			Field	
	15	25	40	60	60		15	25	40	60	60
	Pct	*Pct*	*Pct.*	*Pct*	*Pct*		*Pct.*	*Pct.*	*Pct.*	*Pct*	*Pct.*
2-4	0	3	9	9	6	25-2	3	5	6	14	41
2-33	7	18	26	30	31	25-3	0	2	5	9	24
4-31	7	18	25	32	16	25-4	2	6	9	17	13
4-33	39	41	41	48	28	25-5	9	20	21	23	6
5-32	12	13	14	25	15	26-1	21	30	35	36	21
6-32	20	29	29	36	33	26-2	4	5	8	10	7
8-10	8	16	18	18	43	26-12	5	10	10	19	15
8-12	19	30	33	33	33	27-3	8	16	18	19	12
9-7	5	14	15	19	9	27-12	13	25	28	28	37
15-32	9	13	15	16	37	27-31	8	17	21	22	10
18-31	16	26	29	29	22	29-3	17	33	44	46	34
19-5	9	14	16	16	24	29-4	22	25	25	25	14
19-13	14	27	34	34	36	29-34	6	11	28	28	19
20-13	6	13	17	21	6	32-4	7	11	20	20	11
20-33	0	3	4	12	7	32-10	10	14	15	15	39
22-10	5	14	17	21	32	35-5	6	10	11	20	18
23-30	9	12	17	22	32	37-4	11	18	21	23	7
24-4	10	25	27	30	28	37-18	6	11	14	14	29
24-32	5	8	15	19	24	37-22	0	4	9	18	5
24-34	1	1	2	17	21	38-4	0	6	8	12	42

EFFECT OF NATURAL CONDITIONS ON STIMULATION OF GERMINATION

Data obtained concerning the effects of weathering on the germination of 16 single-plant seed lots are given in table 7. September 25, the first date of collection, represents a period when the seeds might be considered as nearly mature and browning of cones well started. The November and January collections represent seeds from cones which, with the vines, had fallen to the ground and remained in proximity to the soil for approximately 50 and 110 days respectively, being subject to various weather factors. Pertinent phases of the climatic data for the period during which seeds weathered in the field are given in table 8. These may be of further interest in considering the data of germination.

TABLE 7.—*Percentages of germination of single-plant seed lots allowed to remain in the field for 2 periods following maturity* [1]

Plant No.	Germination, in indicated number of days after sowing, of seeds collected—														
	Sept 25					Nov. 25					Jan 25				
	10	25	40	55	70	10	25	40	55	70	5	10	25	35	50
	Pct.	*Pct*	*Pct.*	*Pct*	*Pct*	*Pct.*	*Pct*	*Pct.*	*Pct*	*Pct.*	*Pct*	*Pct*	*Pct*	*Pct*	*Pct.*
2-4	0	0	0	0	0	6	16	16	16	17	26	43	44	44	45
8-12	0	0	0	0	0	0	3	3	3	3	6	11	16	16	16
15-32	0	0	0	0	0	0	1	2	2	2	7	12	26	26	26
18-31	0	1	1	1	2	14	26	26	26	27	2	9	21	23	23
19-5	0	1	1	1	1	1	8	9	9	12	25	37	45	45	46
20-33	0	0	0	0	0	6	12	12	12	12	----	----	----	----	----
22-10	0	0	0	0	0	0	1	1	2	2	11	16	22	23	23
25-4	0	0	0	1	1	2	3	3	4	5	12	16	24	27	28
26-12	0	0	0	0	1	3	4	4	4	4	5	12	13	15	16
27-3	0	0	0	0	0	5	9	9	9	10	10	32	43	43	44
27-31	0	0	0	0	0	2	2	2	2	2	8	9	10	10	11
29-4	0	1	1	1	0	0	9	11	11	11	13	16	21	22	23
38-31	0	0	0	0	0	0	2	2	2	2	7	15	28	29	29
40-33	0	0	0	0	0	0	0	0	0	0	15	31	33	34	34
45-32	0	0	0	0	0	0	0	0	0	0	3	7	24	27	28
47-32	0	0	0	0	0	0	2	2	3	4	6	11	16	19	20
Average	0	0.2	0.2	0.3	0.3	2.4	6.1	6.4	6.6	7.1	10.4	18.5	25.7	26.9	27.5

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[1] Data based on 100 seeds of each sown in greenhouse benches.

TABLE 8.—*Summary of weather data for September to December 1935, and January 1936, at Corvallis, Oreg.*

Month	Precipitation		Temperature			
			Maximum		Minimum	
			Mean	Range	Mean	Range
	Days	*Inches*	° *F*	° *F.*	° *F.*	° *F.*
September	2	1.36	81.7	69-97	51.3	43-59
October	12	[1] 1.95	63.1	36-84	42.6	30-57
November	12	2.26	49.9	36-59	35.5	15-46
December	15	4.76	46.8	39-58	34.3	24-46
January	19	10.82	49.9	44-58	38.1	24-46

[1] 5 inches of snow Oct. 31 not included.

The results show the natural increase in germinating capacity of seeds left to weather in the field and demonstrate the collective influence of the various factors in overcoming dormancy under field conditions. Again variation between lots of seeds from different plants may be noted. In two lots only did seeds collected November 25 fail to germinate better than those obtained September 25, though some differences are small. Seeds gathered January 25 germinated better than those collected November 25 in all lots except one. Seeds from plants 40–33 and 45–32 required longer exposures in the field to induce germination.

DISCUSSION

The results of the experiments have shown that subjection of hop seeds to certain low-temperature treatments exerts a marked beneficial influence on germination percentage. From the data presented it seems probable that longer periods of incubation before storage at −12° C. would have stimulated germination still more. The length of the incubation period seemed to be unrelated to germination following storage at 5°. It also seems likely that periods of refrigeration longer than 5 weeks might have resulted in increased germination.

In the experiment concerned with moisture and temperature treatments, considerable variation in germination existed among varieties. While significant differences were not established, it appears probable that the Red Vine variety might have reacted somewhat differently from the other strains, particularly at storage temperatures of −12° C. More replications would have been desirable to establish variety differences.

The data of table 6 are of special interest in showing wide variations in the germinating habits of seeds from different plants. It should be recalled that hop plants are dioecious. Since the pollination process was uncontrolled, widely different males may have contributed to seed formation in the numerous female plants. Correlation of germination percentages of seed lots from the same plants from year to year would serve to further delineate this character. While treatment of seeds by germinator incubation for 5 days, followed by 5 weeks' storage at 5° C., appeared to be the best of the series used in these experiments, certain seed lots from various female seedlings used in breeding might respond better to other conditions.

From the standpoint of progress in breeding investigations, subjecting seeds derived from controlled pollination to periods of low-temperature storage shortly after maturity allows for greater latitude in utilization and study and more careful control of the material than awaiting germination out of doors. Germination in the former instance may also be more rapid and more complete.

The effects of the treatments on growth after germination were not carefully observed. Differences between lots were probably slight, since the seedlings appeared to be extremely uniform in this respect as growth progressed.

SUMMARY

Germination is very low when recently harvested hop seeds are placed under ordinary conditions of moisture and temperature such as are favorable to the germination of seeds of many plants. Hop seeds undergo a period of dormancy upon maturity of the cone, which, under natural conditions, may be overcome by interaction of weather factors. Seeds from individual plants vary greatly in rate and total amount of germination both under natural conditions and after artificial treatments.

No significant differences were established in reaction among varieties.

The relations of moisture, temperature, and variety to seed germination have been studied. Five days' incubation in the germinator followed by 5 weeks' refrigeration at 5° C. gave the highest germination in the treatments used. Many treatment series exceeded the check lots significantly.

In these experiments storage of moist seeds at 5° C. was greatly superior to storage at $-12°$ in stimulating germination. Three-, four-, and five-week storage periods at 5° resulted in significantly increased germination as the length of the period increased.

Generally longer periods of incubation were accompanied by increased germination in the series refrigerated at $-12°$ C. In the effects upon germination, interrelation of periods of incubation in the germinator and length of the subsequent refrigeration periods at 5° was indicated.

In the general study of incubation and refrigeration periods and temperatures, most germination occurred between 2 and 4 weeks after seeding, regardless of treatment. Hop seeds sprouted at 5° C. after 4 weeks' refrigeration at that temperature.

Total germination of seed lots of 196 plants after 2 weeks' refrigeration at 5° C. and after 60 days in field and greenhouse was significantly correlated and not significantly different.

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INHERITANCE OF EGGSHELL THICKNESS IN WHITE LEGHORN PULLETS[1]

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INTRODUCTION

Evidence that individual hens differ with regard to eggshell expressed as a percentage of total egg weight has been available at least since Willard and Shaw (*18*)[3] published data on eggs from Barred Plymouth Rocks, White Leghorns, White Wyandottes, and American Reds. These writers, however, dealt with an insufficient number of birds to establish breed differences with respect to the composition of eggs. Herrasti (*6*) reported brown-shelled eggs to have a higher breaking strength than white-shelled eggs, but the source of the eggs studied was not given, and the method used was inexact. Significant, high correlations between breaking strength and thickness or percentage of shell have since been conclusively established by Romanoff (*14*), Morgan (*9*), and Stewart (*15*). Romanoff also produced evidence of individual bird differences in breaking strength of shells from White Leghorn pullets.

Taylor and Martin (*16*) published data indicating that the ability to form a thick eggshell was controlled in part by hereditary factors. Eggs from a Barred Plymouth Rock flock were significantly lower in percentage of shell than eggs from a White Leghorn flock. Within the White Leghorn flock, families of daughters from two males differed significantly in the percentage of shell in their eggs. Morgan, Mitchell, and Roderick (*11*) and Morgan (*9*), incidental to other considerations, presented data on the percentage of shell in eggs of Barred Plymouth Rock and White Leghorn flocks. Although these writers drew no conclusion in this respect, their data indicate that the flocks of the two breeds used were significantly different with respect to percentage of shell and breaking strength of shell.

Baskett, Dryden, and Hale (*2*) found that the individuality of the hen was the most important factor in determining the breaking strength of the eggshells produced, although no significant differences between White Wyandottes, White Leghorns, and Rhode Island Reds were found. A definite seasonal effect on breaking strength observed was relatively less important. Munro (*12*) has recently published an analysis of a Barred Plymouth Rock flock which shows significantly higher variance between the offspring of different dams than within the progeny of individual dams in the characters of percentage ash of dry shell and percentage ash of total egg. The differences between groups of daughters from individual dams are attributed to differences in genotype.

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[3] Italic numbers in parentheses refer to Literature Cited, p. 396.

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EXPERIMENTAL PROCEDURE

ORIGIN OF SELECTED LINES

In 1932 a preliminary investigation of shell thickness produced by Single-Comb White Leghorn hens of the California Station flock revealed that certain related groups of birds were more uniform with respect to this character than others.

Three full sisters mated to a half brother (band No. F15) in a study of another egg character were found to produce thin-shelled eggs. An older half sister of the above females (common dam) mated to a different and nonrelated male (band No. F19) also produced thin-shelled eggs. These matings were then designated as thin-shelled selections for 1932. Only five female offspring were produced in that year. In 1933 the surviving dams with their daughters and additional related thin-shelled females were mated to a son from one of the three original full sisters and her half brother. In subsequent years no other stock was added in propagating this line, which for the sake of brevity will henceforth be referred to as the thin line.

Also in 1932 a half brother (common sire) of the thin-shelled male F19, was mated to his dam, his three full sisters, and to seven females not related to him but producing thick-shelled eggs. This formed the original thick-shelled mating, and offspring from it will be referred to as the thick line. In 1933 a cockerel from the thick-shelled line mated to his eight full sisters formed the thick-line selection. As in the case of the thin-shelled line, no other stock was added to the thick line in subsequent years.

BREEDING METHODS

TABLE 1.—*Percentage of shell for eggs of breeders and of their offspring in the successive generations*

Generation	Line	Number of birds and shell percentage for—									
		Dams			Daughters				Daughters used as breeders		
		Number	Mean	Range	Number	Mean	Range	Coefficient of variation	Number	Mean	Range
G	Thick	10	9.53	8.65-10.09	37	9.85	8.70-11.02	7.2	8	9.41	8.18-10.29
	Thin	3	7.97	7.27- 8.86	5	9.14	8.55-10.36	6.9	4	8.90	8.76- 9.37
H	Thick	8	9.41	8.18-10.29	38	9.84	9.12-10.72	4.2	6	10.05	9.62-10.34
	Thin	8	8.55	7.61- 9.37	27	9.44	8.65-10.30	4.9	5	8.77	8.65- 8.98
J	Thick	8	9.95	9.44-10.34	36	9.94	8.60-10.94	5.6	7	10.14	9.65-10.59
	Thin	7	8.81	8.65- 9.08	26	9.24	8.12-10.28	5.7	4	8.88	8.18- 9.31
	Thick ♀×thin ♂	2	10.16	10.00-10.32	6	9.65	8.63-10.11	5.8	5	9.56	8.63-10.11
K	Thick	9	10.10	9.62-10.59	28	9.79	8.72-11.15	5.5	------	------	----------
	Thin	5	8.85	8.18- 9.31	25	9.20	8.38-10.31	5.5	------	------	----------
	Thin ♀×thick ♂	5	9.00	8.69- 9.24	21	9.55	8.38-10.34	5.1	------	------	----------
	Thick ♀×thin ♂	5	10.45	9.82-10.94	37	9.91	8.93-10.91	4.4	------	------	----------
	$\frac{\text{Thin ♂}}{\text{Thick ♀}}$ ♀×thin ♂	5	9.56	8.63-10.11	37	9.51	8.19-10.63	7.2	------	------	----------

In subsequent years full brother by sister inbreeding was avoided wherever possible in mating both thick and thin lines. Families of daughters producing respectively thick- or thin-shelled eggs were mated to their half brothers or to their sires. Throughout the course of the experiment families showing great variability in the shell thickness of the daughters were eliminated in the selection of breeders for further matings. The mean and the range of shell percentage for each year's matings in both thick- and thin-shelled lines are given in table 1. During 1934 and 1935 in producing the J and K generations, reciprocal crosses were made between the thick- and thin-shelled lines, and in 1935 a backcross of F_1 daughters was made to thin-shelled-line males.

In the progress of this experiment considerable difficulty was experienced in maintaining reasonable vigor under the conditions of inbreeding involved. Hatchability was maintained at a fairly satisfactory level, but rearing mortality was excessive in the last 2 years reported. The average coefficients of inbreeding of the daughters produced in the successive generations reported, expressed in terms of percentage, are shown in table 2.

TABLE 2.—*Average coefficients of inbreeding of daughters produced in successive generations of thick- and thin-shelled lines*

Generation	Thick-shelled	Thin-shelled
	Percent	*Percent*
G	9.84	10.00
H	18.75	7.01
J	26.85	20.94
K	30.08	28.33

DETERMINATION OF SHELL CHARACTERS

A uniform procedure was used throughout the experiment in obtaining data on shell thickness and on percentage of shell. Beginning in December and extending through January and February, eggs laid by all pullets in the selected lines were weighed and the shell and shell membranes removed and dried for 24 hours at 100° C. and then weighed. The shell thickness was measured by a micrometer at four or more points ranging from the blunt to the pointed end of the egg, the average value being recorded in terms of hundredths of a millimeter. All the birds from both lines were maintained each year in the same houses, subject to the same management. They were given access to the same ration and kind of supplemental calcium as well as to the same conditions of sunshine. A sample of 10 eggs from each bird was used to calculate average individual percentage of shell and shell thickness. This was found to be an adequate measure of the individual phenotypes, since analyses of variance for each character studied showed uniformly significantly greater variance between means of individuals than between the eggs produced by each individual. Minor variations were introduced in the rations fed from year to year, but in no instance did this factor play any determinable role with regard to the character of shell produced.

STATISTICAL METHODS OF ANALYSIS

Data obtained in the course of the 4 years of study were subjected to a series of statistical analyses. With regard to these, five points in the procedure should be explained.

1. The relation between the amount of shell and egg size can be expressed in several ways. The most common is the percentage of shell or the weight of the shell per unit weight of the whole egg.

Another method, proposed by Hendricks, Lee, and Godfrey (*5*), is that the amount of shell be measured with respect to the unit area of the egg surface, rather than its weight. An index of shell thickness on this basis would be given by the ratio of shell weight to the two-thirds power of the weight of the egg less the weight of the shell. This method is extremely laborious in application when individual eggs rather than means of groups of eggs are to be considered.

A third possible method is one that takes advantage of the allometric relation between a part and the whole, expressed in the formula $y=bx^{\alpha}$. In the terminology of Huxley and Teissier (*7*) α is the limiting equilibrium constant, determining the total amount of shell in an egg of a given size. The principle underlying this method may be interpreted in the same manner as that of the shell-thickness index of Hendricks, Lee, and Godfrey. The essential difference between these methods lies in the fact that while the latter proposes a functional relation between two parts of the whole, the ordinary allometric equation involves the relation between one part and the whole. The error introduced in the ordinary allometric concept is due to the spurious inclusion of the part y in the whole x. In the case of the shell weights under consideration this error is negligible, as is illustrated by the comparison of indices obtained in several groups of eggs as shown in table 3.

TABLE 3.—*Extent of error introduced in the ordinary allometric concept when utilized in determining relation between the weight of shell and egg weight for several samples of eggs*

Group No.	$\frac{\text{Weight of shell}}{\text{Weight of contents}^{2/3}}$	$\frac{\text{Weight of shell}}{\text{Weight of eggs}^{2/3}}$	Difference
TkG1	0.399	0.374	0.025
TkG2	.387	.362	.025
TkH1	.395	.367	.028
TkH2	.391	.365	.026
TkJ1	.398	.369	.029
TkJ2	.399	.373	.026
TkK1	.389	.363	.026
TkK2	.386	.363	.023

Although the values of the indices differ, the difference is consistent throughout, the deviations being maximized by the use of only three decimal places in the computations.

In general the advantage of these two methods over percentage of shell would lie in the fact that an exponential or logarithmic relation may logically exist between egg weight and shell weight, rather than a simple linear arithmetic one implying the identity of the limiting equilibrium constant α with unity. If in reality α differs greatly from unity, the correlation between egg weight and shell weight will be nonlinear and lower than the correlation between the logarithms of these variables. In an effort to learn whether or not the usage of percentage of shell in the statistical analyses proposed would lead to serious errors, comparable coefficients of correlation for several samples of eggs were computed, with the results shown in table 4. The spurious nature of the relation involved will be referred to later (p. 390).

For all practical purposes the two series of coefficients are identical. This would argue for the permissibility of using percentage of shell as the measure of amount of shell. The particular utility of this, in addition to simplifying computation, lies in the fact that the program of selection of the thick and thin lines was based on percentage of shell rather than on any of the other measures. Furthermore, it enables the calculation of the spurious relation between the weight of the part (shell) and the weight of the whole (egg) by the method of Pearl (*13*) for index correlations.

TABLE 4.—*Comparable coefficients of correlation between egg weights and shell weight for several samples of eggs as calculated directly and by use of logarithms*

Group No	Correlation coefficients between—	
	Egg weight and shell weight	Log egg weight and log shell weight
Tk1	0.762	0.750
Tk2	700	.706
Tn1	780	771
Tn2	754	.753

2. The rate of change in egg weight and shell weight from position 1 to position 2 in a clutch can be calculated either by ordinary percentage decrease or increase or on the basis of the continuous compound-interest formula $\log_n W'_2 - \log_n W'_1$. The latter procedure was adopted in view of the fact that successive negative increments in egg weight during a single clutch were found to decrease rather than to be maintained at the same level.

The data in table 5 indicate that the rate of decrease in egg weight or in its constituents is the function to be dealt with rather than the absolute decrease in relation to the original weight. Hence, in the calculation of the rate of change from position 1 to position 2, the difference between the natural logarithms of egg weight or of shell weight were used. The ratio of the latter over the former indicates the rate of decrease in shell weight relative to the rate of decrease in egg weight.

3. The coefficients of correlation were calculated in accordance with the method of Wallace and Snedecor (*17*), their table 16 being used for standards of significance.

4. Gross, spurious, and net coefficients of correlation where percentage of shell is involved were computed, as mentioned, by Pearl's method. The amount of spurious correlation when egg weight and shell weight were correlated was, however, calculated from the formula cited by Davenport and Ekas (*4, p. 90*).

5. Standard errors are used throughout in preference to probable errors.

TABLE 5.—*Rate of change in egg weight from position 1 to position 4 in a clutch*

Weight of first egg in clutch	Clutches	Decrease in weight between positions		
		1 and 2	2 and 3	3 and 4
Grams	*Number*	*Grams*	*Grams*	*Grams*
45.1–50.0	12	0.833	0.592	0.517
50.1–55.0	8	2.575	.625	–.475
55.1–60.0	11	2.109	.882	.009

EXPERIMENTAL RESULTS

STATISTICAL CONSTANTS FOR EGG WEIGHT AND SHELL CHARACTERS

The interrelations of four variables were investigated in this study: Egg weight, shell weight, shell thickness, and percentage of shell. Statistical constants for the variables are presented in table 6, showing the means with their respective standard errors and the coefficients of variation for the four generations of the thick and thin selections. A further subclassification of each generation is made on the basis of the position of the egg in the clutch. As will be shown later (p. 392), the thick and thin lines differ in their behavior with respect to the changes observed in the eggs laid in different positions in the cycle. Hence position 1 and position 2 of each clutch are treated here separately. A slight discrepancy in the number of birds involved will be noted between table 1 and table 6. This discrepancy occurs because the determinations of shell percentages on which the breeding selections were based were obtained irrespective of clutch position. Hence a few birds which laid in one-egg cycles or in very long cycles are included in table 1 but not in table 6. On the other hand a number of birds laid several two-egg clutches and are included in table 6, but not in table 1, because their total production in the period of the annual recording of data was less than 10 eggs, owing to pause, late maturity, or mortality. It was considered that no representative individual percentage of shell could be determined for such birds, yet the complete two-egg or larger clutches they produced could be included in the general population analysis. Because of the fact that the selected lines did not excel in production and because only a maximum of 10 determinations was made for each bird, only positions 1 and 2 in the clutch were adequately represented in the data.

Data on the coefficients of variation for egg weight and shell characters according to clutch position for the various generations of the two selected lines show that, except for egg weight in the thick-shelled line, no uniform trend of reduction in variability of these characters with successive generations of selection and inbreeding is apparent. This may be interpreted as indicating either that no pronounced increase in homozygosity was effected for these characters in either line during the generations of selection reported or that variability controlled by heredity in these characters is obscured by the variability normally found within the production of individual birds. Further, the two lines are quite similar in the degree of variability of the characters considered. In table 1 the coefficients of variation of the daughters' means with respect to percentage of shell are given. Here some reduction of variability is apparent between the first and second generations of both lines. In later generations variability increased slightly over that of the second generation. Table 7 presents an analysis of the significance of differences between the means of the thick- and thin-shelled lines for the characters reported in table 6. The two selected lines were significantly different beginning with the first generation for the characters of shell weight and percentage of shell. Except for shell thickness of eggs laid in position 1, G generation, the same was true for shell thickness. In the last three generations the egg weight also differed significantly between the lines.

Table 6.—*Statistical constants for all eggs produced in first and second clutch positions by the thick- and thin-shelled lines*

Line and generation	Clutch position	Birds	Eggs	Means[1]				Coefficients of variation			
				Egg weight	Shell weight	Shell thickness	Percent shell	Egg weight	Shell weight	Shell thickness	Percent shell
		Number	*Number*	*Grams*	*Grams*	*Millimeters*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Thick:											
G	1	36	101	52.50±0.47	5.23±0.055	0.359±0.0029	9.96±0.062	8.93	10.52	8.08	6.22
	2	36	101	50.23± .46	4.92± .051	.348± .0025	9.79± .064	9.28	10.37	7.18	6.54
H	1	43	99	53.43± .40	5.21± .040	.368± .0023	9.76± .056	7.47	7.68	6.25	5.74
	2	43	99	50.68± .39	5.00± .038	.366± .0021	9.88± .058	7.60	7.60	5.74	5.88
J	1	30	79	52.75± .41	5.21± .048	.377± .0024	9.89± .072	6.90	8.25	5.57	6.48
	2	30	79	50.22± .36	5.07± .046	.376± .0028	10.10± .075	6.29	8.09	6.65	6.63
K	1	22	52	49.86± .43	4.94± .072	.368± .0049	9.90± .118	6.16	10.53	9.51	8.07
	2	22	52	48.19± .50	4.79± .064	.368± .0037	9.96± .104	7.41	9.60	7.34	7.55
Thin:											
G	1	5	12	50.94±1.01	4.82± .118	.346± .0061	9.46± .202	6.87	8.51	6.07	7.40
	2	5	12	49.17±1.02	4.59± .136	.328± .0055	9.34± .199	7.20	10.24	5.79	7.40
H	1	28	80	49.11± .37	4.67± .040	.347± .0022	9.51± .065	6.72	7.71	5.76	6.10
	2	28	80	47.39± .37	4.38± .045	.335± .0026	9.26± .075	7.07	9.13	6.87	7.25
J	1	20	58	50.63± .40	4.69± .046	.348± .0028	9.27± .075	5.96	7.46	6.03	6.16
	2	20	58	48.71± .37	4.52± .045	.347± .0026	9.30± .079	5.81	7.52	5.76	6.47
K	1	18	56	45.92± .51	4.32± .080	.346± .0036	9.39± .099	8.30	13.89	7.80	7.86
	2	18	56	44.60± .45	4.10± .055	.334± .0033	9.20± .082	7.58	10.00	7.49	6.64

[1] Standard errors, not probable errors, are used.

TABLE 7.—*Differences between thick- and thin-shelled lines for eggs produced in the first and second clutch positions*

[Thick line minus thin line]

Generation	Clutch position	Egg weight [1]	Shell weight [1]	Shell thickness [1]	Percent shell [1]
		Grams	*Grams*	*Millimeters*	*Percent*
G	1	[2] 1.56±1.11	0.41±0.130	[2] 0.013±0.0068	0.50±0.211
	2	[2] 1.06±1.12	.33± .145	.020± .0060	.45± .209
H	1	2.32± .55	.54± .057	.021± .0032	.25± .085
	2	3.29± .54	.62± .058	.031± .0033	.62± .095
J	1	2.12± .57	.52± .066	.029± .0037	.62± .104
	2	1.51± .52	.55± .064	.029± .0038	.80± .109
K	1	3.94± .67	.62± .108	.022± .0061	.51± .154
	2	3.59± .67	.69± .084	.034± .0050	.76± .132

[1] Standard errors, not probable errors, are used

[2] Not significant.

These data show that, effective from the first generation of selection, significant differences in amount, thickness, and percentage of shell were obtained. Yet there is little evidence of any effect of the further selection of the type used in decreasing or increasing respectively the amount of shell, shell thickness, or percentage of shell in the two lines. The method of selection employed was successful in segregating thick- and thin-shelled lines, but not in modifying the expression of the shell characters within either line.

CORRELATION COEFFICIENTS BETWEEN EGG WEIGHT AND SHELL CHARACTERS

Table 8 presents coefficients of correlation between the different variables involved. Since shell weight is a part of egg weight and percentage of shell is an index of the two, gross correlations between these variables include a certain amount of spurious correlation. By subtracting the spurious part from the gross, the net coefficients of correlation are obtained. All of these appear in table 8 for each generation, each line, and each clutch position.

Though correlation coefficients in each column vary considerably in magnitude, general trends may be noted. With the exception of the

TABLE 8.—*Correlation coefficients between egg weight*

Line and generation	Clutch position	Eggs	Egg weight / Shell thickness	Shell weight / Shell thickness	Shell thickness / Percent shell
		Number			
Thick:					
G	1	101	0.327	0.721	0.745
	2	101	.203	.660	.778
H	1	99	.049	.583	.740
	2	99	.039	.603	.765
J	1	79	−.060	.628	.854
	2	79	.019	.740	.887
K	1	52	.291	.814	.855
	2	52	.123	.755	.823
Thin:					
G	1	12	−.052	.653	.849
	2	12	.067	.381	.468
H	1	80	.163	.761	.802
	2	80	.111	.750	.982
J	1	58	−.011	.723	.915
	2	58	−.071	.671	.844
K	1	56	.584	.834	.755
	2	56	.246	.761	.860

correlations between egg weight and shell thickness, most of the coefficients reported are statistically highly significant. The majority of exceptions to this lie in the G generation of the thin line, where only 12 pairs of eggs are represented. The correlations between shell weight and shell thickness (with the exception of the G generation, position 2, thin line) lie in the range between 0.583 and 0.834 and do not appear to depend on the origin of the sample. Similarly, the correlation coefficients between shell thickness and percentage of shell (with the same exception) range from 0.740 to 0.982 and are higher than those between shell weight and shell thickness in the previous column with the single exception of the K generation, thin line for position 1. Thus, while to a great degree, shell thickness is independent of egg weight, it seems to be adequately represented by percentage of shell and somewhat less so by the weight of shell.

The gross coefficients of correlation between egg weight and shell weight range between 0.558 and 0.820 and are again independent of the origin of the sample. These values are of the same general magnitude as those presented by previous investigators, such as 0.541 reported by Curtis (*3*), 0.644 by Jull (*8*), and 0.636 by Asmundson (*1*). The amount of spurious correlation is approximately 0.100 in all of the samples.

The index correlations between percentage of shell on the one hand and egg weight and shell weight on the other are of considerable interest. While the gross values do not show consistent significance, the elimination of the spurious correlations tends to change the picture. Thus percentage of shell and egg weight show in general a significant positive net correlation, varying in magnitude from 0.280 to 0.875. On the other hand the gross correlations between shell weight and percentage of shell are transformed from a low positive to a low negative net coefficient.

Evidently egg weight has a greater influence on the percentage of shell than has shell weight. While the increase in egg weight increases the percentage of shell, the increase in shell weight operates in the opposite direction. This, of course, applies to populations varying simultaneously in both components of the index, since obviously the reverse is true wherever one of the two components is constant.

and shell characters for thick- and thin-shelled lines

Egg weight / Shell weight			Egg weight / Percent shell			Shell weight / Percent shell			r at	
Gross	Spurious	Net	Gross	Spurious	Net	Gross	Spurious	Net	$P=0\ 05$	$P=0\ 01$
0.809	0 100	0 709	−0 068	−0 647	0 579	0 275	0 647	−0 372	0 195	.254
745	098	.647	−.219	− 667	448	.334	.667	−.333	195	254
707	.098	609	− 351	− 697	346	.377	.697	−.320	195	.254
.713	.099	.614	− 379	− 707	328	379	.707	− 328	195	254
647	.099	548	− 241	− 641	.400	376	641	− 265	.217	283
.599	101	498	−.217	− 805	.588	380	.805	− 425	217	.283
665	099	566	− 106	− 689	583	.261	689	− 428	273	.354
619	099	520	−.191	−.611	420	368	.611	− 243	273	354
.605	.095	.510	−.246	− 628	.382	388	.628	−.240	576	.708
.663	093	.570	− 054	− 575	.521	316	575	− 259	576	.708
.663	.095	.568	−.268	−.548	280	.378	548	−.170	217	283
669	.092	.577	−.140	− 612	472	341	612	−.271	217	.283
.616	092	.524	−.226	−.624	.398	379	624	− 245	.250	325
.558	.093	.465	−.250	−.612	.362	.398	.612	− 214	.250	.325
.820	.094	.726	.362	−.513	875	.175	513	−.338	273	.354
.739	.092	.647	− 282	−.604	.322	.294	604	− 310	273	.354

EFFECT OF CLUTCH POSITION ON EGGSHELL CHARACTERS

A comparison of mean values for egg weight and shell weight for clutch positions 1 and 2 in both thin and thick lines (table 5) shows that, in every instance, the average eggs of position 2 are smaller and have less shell than those of position 1. The mean values for shell thickness and percentage of shell, however, do not have a similar uniform trend.

The relative percentage decreases in egg weight and shell weight from position 1 to position 2 and the ratio between them for each generation of each line are presented in table 9. In the G generation the percentage decrease of shell weight was greater than that of egg weight in both lines. In subsequent generations, however, the percentage decrease of shell weight in the thick-shelled line was markedly less than the corresponding decrease in egg weight. While considerable variation in the percentage decrease of both egg weight and shell weight took place in the thin-shelled line from generation to generation, in the last three generations the ratio of decrease of shell weight to that of egg weight was at least twice that observed in the thick-shelled line. In these generations the relative egg-weight decreases were greater in the thick-shelled than in the thin-shelled line; the reverse was true of relative shell-weight decreases.

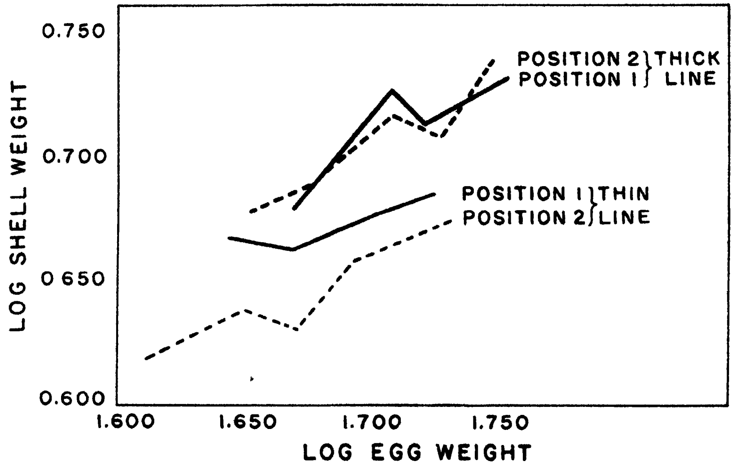

FIGURE 1.—Effect of clutch position on shell weight in relation to egg weight for the thick and thin lines in the H generation.

The graphic presentation of the difference between the thick- and thin-shelled lines for the H generation shown in figure 1, indicates that eggs from clutch position 2 of the thin-shelled line have uniformly less shell than those from position 1 for the entire range of egg weight observed. On the other hand, in the thick-shelled line the eggs of clutch position 2 were not consistently different from those of position 1 with respect to shell weight in different egg-weight classes. Thus in the thick-shelled line the amount of shell relative to egg weight is maintained at approximately the same levels in positions 1 and 2, while in the thin-shelled line there is a pronounced decrease from position

1 to position 2. The K generation gives results similar to those of the H generation. In the J generation, however, the eggs of clutch position 2 in the thin-shelled line maintained an approximately equal relative decrease in egg weight and shell weight, while those from the thick shelled line had less relative decrease in shell weight and showed an increased percentage of shell (table 9).

TABLE 9.—*Rate of decrease* [1] *of egg weight and shell weight from position 1 to position 2 in a clutch for thick- and thin-shelled lines*

[Means of hen means with 3 or more clutches]

	Thick-shelled line				Thin-shelled line			
Generation	Hens	Egg weight [2]	Shell weight [2]	Ratio	Hens	Egg weight [2]	Shell weight [2]	Ratio
	Number	*Percent*	*Percent*		*Number*	*Percent*	*Percent*	
G	23	4.67±0.74	6.59±1.01	1.41	3	3.68±1.22	4.77±0.92	1.30
H	17	6.01± .74	4.08±1.04	.68	18	3.71± .62	6.29± .92	1.70
J	13	5.34± .51	2.56± .78	.48	14	3.43± .71	3.36±1.17	.98
K	7	4.37±1.38	2.21±2.62	.51	12	2.76± .67	3.73±1.22	1.35

[1] The formula $\log_e W_2 - \log_e W_1$ was used to calculate the rate of decrease.

[2] Standard errors, not probable errors, are used.

Although the above results indicate that the thick-shelled line was able to maintain the relative amount of shell in eggs of clutch position 2 better than the thin-shelled line, individual differences in the relative amount of shell in clutch positions 1 and 2 occurred in both lines in all generations. Obviously, the kind of selection practiced was only partially effective in differentiating lines varying in ability to form relatively as heavy shells for eggs of clutch position 2 as for position 1.

RESULTS FROM CROSSES BETWEEN LINES

Data for average percentage of shell are given in table 1 for reciprocal F_1 generations and for a backcross of F_1 females to thin-shelled-line males. Differences in percentage of shell for the K series between the various crosses and the selected lines are given in table 10.

The thick-shelled line is significantly higher in percentage of shell than the F_1 of thin female × thick male or of the backcross of F_1 $\frac{\text{thin ♂}}{\text{thick ♀}}$ female × thin male. The thick-shelled line is not significantly different in percentage of shell from the F_1 from thick female × thin male. The thin-shelled line, on the other hand, is significantly lower than all other matings.

There is a significant difference between the reciprocal F_1 generations, the thick female × thin male giving the higher percentage of shell. While the thick female × thin male F_1 generation was significantly higher than the backcross of F_1 $\frac{\text{thin ♂}}{\text{thick ♀}}$ female × thin male, the differences between the reciprocal F_1 generation and this backcross are not significant.

Unfortunately, for purposes of clear comparison, the F_1 thick female × thin male dams produced in the J generation and mated to thin-shelled-line males to give the backcross in the K generation had a lower average percentage of shell than the same F_1's produced in the K generation. This may have been a factor in producing the significant difference between the F_1 thick female × thin male and the backcross F_1 $\frac{\text{thin}\,♂}{\text{thick}\,♀}$ female × thin male, as given in table 10. All the difference between these two generations cannot safely be attributed to the influence of the thin-shelled-line males used in the backcross.

TABLE 10.—*Differences in percentage of shell*[1] *for the K generation*

[Ordinates minus abscissae]

Item	Thick ♀ × thin ♂	$\frac{\text{Thin}\,♂}{\text{thick}\,♀}$ ♀ × thin ♂	Thin ♀ × thick ♂	Thin
Thick	[2]−0.12±0.085	0.28±0.133	0.24±0.100	0.59±0.098
Thick ♀ × thin ♂		.40± .123	.36± .086	.71± .085
$\frac{\text{Thin}\,♂}{\text{Thick}\,♀}$ ♀ × thin ♂			[2]−.04± .134	.31± .133
Thin ♀ × thick ♂				.35± .100

[1] Standard errors, not probable errors, are used.
[2] Not significant.

Unfortunately in the J generation no offspring were secured from a attempted thin female mating × thick male; hence the reciprocal backcross in the K generation could not be made.

Because of the differences in egg weight between the thick- and thin-shelled lines (table 6), it may be suggested that the shell percentage depends on egg size not only within lines as shown by the net correlation coefficients in table 8, but also between lines. However, that such is not the case can be demonstrated:

(1) By figure 1, where percentage of shell differed between the two lines in eggs of the same weight, and (2) by the fact that, while the difference in percentage of shell between the reciprocal crosses was significant (table 10), the mean egg weights for the thin female × thick male and the thick female × thin male offspring were respectively 48.79±0.65 and 48.95±0.67 g.

DISCUSSION

A segregation of two lines of chickens differing significantly in amount, thickness, and percentage of eggshell based upon phenotypic selection of females and mating with related males was obtained in one generation and continued for three further generations. The evidence that the shell character of the dam was of extreme significance in determining that of the female offspring, as revealed by the results from reciprocal F_1 generations, explains why the two lines became significantly different so readily despite the relationship between sires used in establishing the two lines.

A genetic explanation of the above results cannot easily be deduced. Since eggshell characters are sex-limited in their expression, no phenotypic classification of the males, such as formed the basis of selection of the females, was possible. The comparative impotence of some of the thin-line males in affecting the shell characters of their daughters in the above experiment might indicate either (1) that the greater number of them were heterozygous for genes controlling the type of eggshell produced or (2) that they were less influential than the dams in determining the phenotype of the daughters. An interpretation of the results presented in table 10 on the basis of a maternal effect would not be justified in the absence of experimental evidence disproving the first alternative suggested above.

A method of selection of breeding females which disregards the effect of differences in inherent rate of production on eggshell characters seems to be limited in its results. Although in this work it seemed to produce lines with significantly different percentages of shell, further generations of the same type of selection gave no greater differentiation between the lines or any apparent increase in homozygosity, which would be shown by decreased variability of the expression of shell character. Since the discovery of the differential effect of clutch position in the two lines, it is possible that two factors affect the shell percentage of an individual: (1) The amount of shell characteristically produced for the first egg of the clutch and (2) the ability of the individual to maintain the shell thickness and percentage in subsequently produced eggs of the clutch. If these are valid, separable factors, the failure to secure greater uniformity in the lines selected for four generations can be readily understood.

A selection of potential breeding females for relatively high percentages of eggshell during the winter months of the pullet year of production does seem to be practicable. In these months, when according to Baskett, Dryden, and Hale (*2*) a relatively uniform strength of shell is maintained, approximately 10 percent of the total egg weight as eggshell may be recommended as a desirable qualification for a breeding female.

The physiological implications of hereditary conditions affecting the metabolism and efficient utilization of calcium in the hen cannot be ignored (*12*). The meager evidence available on calcium balance in the laying hen (*10*) indicates that there are individual differences between birds in ability to maintain a positive calcium balance and an efficient output of calcium as eggshell. The possibility that hens which show a relatively high decrease in amount and proportion of eggshell with successive eggs of a clutch may be in some way inefficient in calcium metabolism should not be overlooked. The interrelation between hereditary, nutritional, and physiological factors influencing the character of eggshell needs to be determined.

SUMMARY

A selection of hens producing thin- and thick-shelled eggs, respectively, and a subsequent breeding program of mating with closely related males were effective in establishing two lines of chickens producing significantly different types of eggshell.

Continued selection of this type was ineffective in further differentiation of the two lines or in decreasing the variability of expression of eggshell characters in subsequent generations.

Analysis of the data indicated that heritable factors were involved in the determination of the amount, thickness, and percentage of eggshell characteristic of individual hens. In several matings the dams seemed to have relatively more influence in determining the character of shell produced by the daughters than the sires.

The females of the thick-shelled line maintained the ability to produce eggs with relatively little or no decrease in shell percentage from clutch positions 1 to 2. The pullets of the thin-shelled line, however, showed a greater tendency toward lower shell percentages in eggs in position 2. Individual variations in this respect were found in both lines.

The possible relation between hereditary factors and the nutritional requirements and physiological efficiency of laying hens with respect to calcium metabolism and balance is pointed out.

In the treatment of data on the populations available for this study, the percentage of total egg weight represented by eggshell was found to be as accurate an expression of individual variation as more cumbersome formulas which take into account the effect of differences in total egg weight upon shell weight.

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CHEMICAL ELIMINATION OF SAPROPHYTES DURING LABORATORY GERMINATION OF SEED PEAS[1]

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INTRODUCTION

The appraisal of the viability of a seed stock may be seriously handicapped by the presence of fungi and bacteria. Undeniably it is advantageous to determine the percentage of seeds actually infected by, or merely contaminated with, micro-organisms, for whether they be pathogens or saprophytes they depreciate the planting value of the seed. But it is essential in determinations of live seeds, and especially in comparing vigor of seedlings, that the micro-organisms be confined to their points of origin.

Perhaps the most instructive method of germination consists in placing the seeds widely apart on flat towels. The growth of the fungi and bacteria can be witnessed, yet at the same time the clean seeds are not contaminated by their neighbors during the germinative period. For the evaluation of viability, vigor, and associated micro-organisms this would be very exact. A spatial method of quarantine or isolation cannot be continuously practiced in routine germination work since sufficient space is not available. To maintain a high relative humidity and to conserve room in the germinator pea seeds (*Pisum sativum* L.) must be placed relatively close together on paper towels which are folded in loose rolls. Under these conditions fungi associated with a few seeds may spread over a part or all of a roll either by contact with adjacent seeds or seedlings or by growth of mycelium through the towel. Bacteria not only decompose the infected cotyledons, but also destroy the sprouts, and may weaken or kill surrounding normally strong seedlings. Decomposed radicles or plumules frequently lead to uncertain or incorrect readings, since it is often impossible to decide whether the growth of small rotted seedlings was naturally slow or was inhibited by saprophytes.

The mere presence of profuse mycelial growths, as illustrated by Koehler and Holbert (*12*)[3] for corn (*Zea mays* L.), is undesirable, even though no necrotic injury occurs. Manual handling of the tests is rendered unpleasant, and unless the obscuring fungus masses are removed the examinations are incomplete and the results uncertain. Freshly harvested mature seed stocks rarely prove troublesome. The small-seeded varieties of garden peas and all field peas usually harbor fewer fungus and bacterial associates than do the large-seeded varieties. Carry-over or storage stocks present more difficulty, roughly in propor-

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[3] Italic numbers in parentheses refer to Literature Cited, p. 422.

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tion to the percentage of dead or decadent seeds. Mixed lots or stocks resulting from indiscriminate blending of peas from returned packets or other small containers are commonly carriers of storage molds.

Tests in soil, while preferred by some laboratories on the basis of similarity to actual field conditions, are not without serious disadvantages. Unless the soil has a low inoculum potential the germination results may be an indication or measure of the soil condition rather than of the seed vitality. Moreover, examinations of the seeds for saprophytes on pathogens must be foregone.

On the thesis that chemicals which protect seeds in the soil should prove protective in germinator tests, the authors made a study of a number of dusts and dips to determine which would prove most effective in eliminating saprophytes during the germination of seed peas in the laboratory. The results of this study are reported in the present paper.

REVIEW OF LITERATURE

Observations of samples of germinating barley, cabbage, corn, and peas that had been treated for field planting strongly indicated that several materials were effective but more toxic in towels than in the soil. Horsfall and his coworkers (*7*) in their studies with seeds treated with cuprous oxide showed that injury occurs in sandy soil and clay subsoil. Porter (*13*) reported that a dust compounded from New Ceresan (5-percent ethyl mercury phosphate) and talc was very valuable in his germination studies. Treated and nontreated samples of cereals germinating in the seed laboratory of the New York State Station showed that, on these seeds at least, the organic mercurials, and, to a lesser extent, certain copper compounds prevented the development of *Alternaria* spp., *Rhizopus nigricans* and *Penicillum* spp. The internally-borne *Helminthosporium* spp. of barley seed were not controlled by several copper compounds but were controlled by organic mercurials (*4*). Christensen and Stakman (*2*) stated that Ceresan (2-percent ethyl mercury chloride) effected practical control of seedling blights of barley. Bucha (*1*) suggested the laboratory possibilities of Ceresan by emphasizing its value in obtaining clean accurate tests of cottonseed. Hofer and Hamilton (*6*) in surface sterilizing legume seeds found an 0.006-percent concentration of ethyl mercury phosphate very effective.

EXPERIMENTAL MATERIALS AND METHODS

No attempt was made to secure highly contaminated lots of pea seed. The samples submitted for routine germination studies or those collected by inspectors from open stocks were utilized whenever possible. These varied from the finest current stock offerings of western seed growers to the blended contents of commission-box packets, or in other words, from very clean to severely infested seed. The germination of an untreated portion, usually 100 seeds or more, was compared with that of one or more treated portions depending on the size of the sample available. Seedling emergence from greenhouse soil was rarely used as an indication of the reliability of chemical protection. All laboratory portions were germinated at 20° C. in moistened paper towels, 10 by 14 inches, folded into loose rolls of about 1½-inch diameter. The rolls were unfolded after 6 days for examination of the seedlings. Seed producing either strong, normal sprouts and weak sprouts which appeared to have been developing normally prior to microfloral attack or to chemical injury were counted as germinated. The degree of contamination and rotting was observed and usually recorded as the percentage of seeds injured by, or in contact with, any pathogen or saprophyte. The general condition of the treated lots was compared with that of the control. Particular attention was paid to acceleration of growth, length of plumules and radicles, development of secondary and vesicle roots, and presence of chemically induced abnormal seedlings. As a statistical basis for comparison of development, the radicles and plumules of the seedlings were measured in some experiments while in many others the entire portion (100 seeds and seedlings) was weighed.

Materials known to be effective in control of smut, seedling rots, damping-off, storage decay, and other diseases were requested from the manufacturers. These were applied initially as recommended by plant pathologists or by the manufacturers, but several were later altered to change the rate of adherence. The following chemicals were used:[4]

Mercury compounds:
  Volatile: 2-percent ethyl mercury chloride (Ceresan); 1-percent ethyl mercury phosphate (New Semesan Jr.), 5-percent ethyl mercury phospate (New Ceresan), and 30-percent hydroxylmercurichlorophenol (Semesan).
  Nonvolatile: 8-percent ethyl mercury cyanamide (Barbak C), mercuric chloride, mercurous chloride, a 3-percent metallic mercury dust (Merko), 2-percent ethanol mercury chloride (Sanoseed), and phenyl mercury nitrate.
Copper compounds:
  Very soluble: Basic and monohydrated copper sulphate.
  Slightly soluble—copper carbonate (25-, 50-, and 52-percent copper content), copper hydroxide, copper oxalate, copper oxychloride, copper phosphate, copper stearate, cuprous oxide, and a dusting copper of unknown composition.
Other materials:
  Zinc: Zinc chromate, zinc oxide, zinc stearate, and mixed zinc compounds (Vasco 4).
  Aldehydes: Formaldehyde dusts (Ansul, Corona, and Smuttox), and methyl aldehyde plus copper and mercury (Grainaide).
  Miscellaneous: Ammonium sulphate, borax, copper-lime-arsenite, ferrous sulphate, potassium dichromate, potassium iodide, and dusting sulphur.

The chemicals were applied as dusts by agitating the seeds with the chemical in an Erlenmeyer flask or a fruit jar. The excess chemical was removed by shaking the seeds in a tea strainer. The seeds were either immediately placed on towels, planted in the soil, or held in tight paraffin containers for 48 to 72 hours before the beginning of germination. A wet or dip method proved easier in practice in that the seeds were simply dropped into a beaker of the suspension or solution of the test chemical, collected by pouring the liquid and seeds into a tea strainer, and immediately spaced on the towels. This method was altered infrequently; the dry seeds were placed in a tea strainer, and liquid was poured over them once or twice. Usually the working portions of both the dusts and dips were used continuously until exhausted. The chemically treated samples were always compared with control samples and germinated under identical conditions. Because of the limited size of the seed stocks, machine treatments with a determined weight and volume of chemical were impractical. However, numerous samples were drawn from cuprous oxide- and graphite-dusted bulks. The microflora and the vigor of the seedlings were regularly noted and compared with untreated seeds of the same variety.

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[4] The cooperation of the manufacturers in supplying these chemicals is gratefully acknowledged.

EXPERIMENTAL RESULTS

DUST TREATMENTS

The majority of the chemicals employed in this study were processed for use as dusts. In treating large quantities of seeds dry applications are obviously preferable to dip or soak treatments. In order to secure results which would be comparable to control of micro-organisms on a commercial basis the first experiments in the laboratory were performed with commercially available dusts.

The recommended procedure of agitating the seeds and chemical for a period of 5 to 10 minutes was not practical in the routine laboratory studies. Moreover, certain preliminary trials with shaking periods of 30 seconds to 10 minutes showed that fungus control was no better for the 5-to-10-minute than for the 30-to-60-second treating period. Dilution of the manufactured product by an inert material was necessary with several chemicals in order to reduce the injury to the pea sprouts. Usually, however, the maximum amount of dust adhering to the dry seeds closely approximated the recommended dosage of 2 to 2½ ounces per bushel of seed, and the addition of a diluent to the chemical was not required.

Ethyl Mercury Phosphate

Since routine germination tests with treated cereals had repeatedly proven that certain mercurials would prevent the discoloration of seeds by *Alternaria* sp. and *Helminthosporium* spp., these materials were studied extensively as treatments for the larger agricultural seeds. Porter (*13*) suggested the use of a 1-percent ethyl mercury phosphate dust for protecting corn sprouts. The dust, made by blending one part of New Ceresan with four parts of French talc, is equivalent to the commercially compounded product, New Semesan Jr., which is widely applied to sweet- and field-corn seed. Porter found that the use of diluted New Ceresan expedited the reading of germinated corn because both saprophytes and pathogenes were eliminated. Cuprous oxide did not prove satisfactory.

Koehler (*10*) reported that the recommended dosage of New Ceresan (one-half ounce per bushel) for oats was probably heavier than actually required for smut control. In fact, a ⅜-ounce dosage appeared effective, even through the seed was planted before the mercury could volatilize. Later he (*11*) observed that cuprous oxide protected crown-injured corn seed better than did the mercury compounds, but in the control of seed-borne organisms, the opposite was true.

Under the assumption that approximately 2¼ ounces of talc would adhere to a bushel of pea seeds, a mixture of one part of New Ceresan with five parts of talc was prepared in order to obtain a per-bushel dosage equivalent to three-eighths of an ounce of the undiluted chemical. A mixture containing 0.833 percent of ethyl mercury phosphate was used in several thousand germination tests with results that were uniformly satisfying. When seeds are shaken with the dust in a flask the dosage per bushel naturally varies with the size, moisture content, surface characters of the seed, and to some extent with the period of agitation. Onion, radish, and certain flower seeds were injured occasionally, but in general they tolerated comparatively large dosages. Pea seeds, on the other hand, having a low surface volume ratio, were not heavily treated. Presumably the 0.833-percent ethyl mercury phosphate dust unless given time to volatilize might not control fungi, but, as shown in table 1, this dust actually was very effective, allowing no, or seldom a trace of, saprophytes to develop.

Bentonite clay was also used as the inert component in diluting New Ceresan. It appeared to be but little different from talc in adherence.

The content of ethyl mercury phosphate in the dust was further reduced. A mixture containing only 0.455 percent of this organic mercurial proved adequate for practical control. The fungicidal value of this dust was only slightly lower than that of the 1 to 5 mixture while the seedling weight was increased by its use (table 1).

TABLE 1.—*Influence of dilution of New Ceresan dust, or of storage of the treated seeds, on germination and on the saprophytic and bacterial contaminants of pea seed, and on the green weight of, and chemical injury to, the sprouts*

Type or variety of seed and dust used	Period of storage	Germination	Seeds or sprouts contaminated with—				Green weight of sprouts	Chemically injured tests (ratio [4])
			Rhizopus nigricans	*Penicillium* sp.	*Dematium pullulans*	Bacteria		
	Hours	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Grams*	
New-crop bulks								
New Ceresan 1, talc 5 [1]	0	92.7	0	0.1	0	0.5	40.1	4/43
Check	----	91.0	5.1	2.1	11.3	2.4	36.8	0/43
Thomas Laxton								
New Ceresan 1, talc 5 [1]	0	96.8	.1	4	0	4.7	37.8	1/20
Check	-----	90.7	11.9	3.5	16.7	7.9	35.7	0/20
Stored bulks [3]								
New Ceresan 1, talc 5 [1]	0	91.3	1	1	0	1.5	46.7	1/26
New Ceresan 1, talc 10 [2]	0	91.5	3	1	3	1.5	48.0	0/26
Check	---	84.6	30.3	2.4	21.5	8.4	42.4	0/26
Alderman								
New Ceresan 1, talc 5 [1]	0	86.6	.6	1	1	6.8	41.3	0/28
Do [1]	[3] 72	85.0	6	2	0	6.7	40.2	1/28
Check	---	78.7	42.3	7.9	26.4	16.0	36.9	0/28
Thomas Laxton								
New Ceresan 1, talc 5 [1]	0	80.9	6	.5	0	8.8	39.8	0/27
Do [1]	[3] 48	81.2	9	1.3	0	9.6	39.4	0/27
Check	---	72.3	52.2	13.9	11.8	21.7	34.2	0/27

[1] A blended mixture of 1 volume of New Ceresan with 5 volumes of talc

[2] A blended mixture of 1 volume of New Ceresan with 10 volumes of talc

[3] Seeds were stored in tight paraffin containers immediately after treatment.

[4] The numerator is the number of 100-seed tests containing chemically injured sprouts, the denominator s the total number of tests.

DELAYED INITIATION OF GERMINATION OF SEEDS TREATED WITH ETHYL MERCURY PHOSPHATE

Since ethyl mercury phosphate is volatile, the fungicidal action of New Ceresan depends not only upon the dosage used but also upon the time elapsed between treatment and germination. Koehler and Holbert (*12*), Porter and Layton (*14*), and Johnson et al. (*8*) have recommended that smut-infected cereals be treated 1 or 2 days prior to seeding. Probably very few phytopathologists have observed the daily growth of sprouts from treated seeds. Hence they have little knowledge of whether or not a storage period is required to render New Ceresan effective against *Rhizopus nigricans*, *Penicillium* spp., *Alternaria* spp., *Helminthosporium* spp., and other fungi on cereals. Bucha (*1*) commented on the remarkable fungicidal action of Ceresan against fungi commonly contaminating cotton seeds. Apparently a storage period was not necessary. Koehler (10) applied various dosages of New Ceresan to oats and stored the seed from 2 hours to 72 days before seeding. Smut elimination was effected by a ⅛-ounce-per-bushel treatment in a 72-day storage, by ¼-ounce treatment in a 5-day storage, or by ½-ounce treatment in a 2-hour storage.

Porter (*13*) recommended storage of the treated seeds for 24 hours to avoid mercury injury. No mention was made of enhancing the fungicidal action. In order to determine the value of delaying the initiation of germination 55 samples of new-crop western pea seed were treated with New Ceresan by different methods in the New York State Station laboratory. The samples were divided into two portions: One portion was agitated in a flask containing the 1 part New Ceresan to 5 parts talc dust while the other was left as a control. The dusted seeds were divided into 2 parts, one part was placed to germinate immediately, the other stored for 48 or 72 hours before germination. Each lot was removed in exactly 144 hours and the percentage of germinated seeds recorded. The results (table 1) indicate that there is no advantage in delaying germination and also that a short storage period is not conducive to mercury injury.

OTHER VOLATILE MERCURIALS

The proprietary dusts, Ceresan, New Semesan Jr., and Semesan, which also contain volatile mercurials, were found useful as substitutes for New Ceresan. They were applied in the original manufactured forms without reduction with an inert dust. Dilutions to avoid mercury poisoning of the seedlings apparently were not necessary. Injury was noted in a few trials, but undoubtedly it was caused by failure to remove all of the excess dust. These materials, when present in amounts greater than those normally adhering to dry pea seeds, were toxic and caused abnormal growth of the seedlings.

TABLE 2.—*Influence of certain proprietary dusts and mercury compounds on the germination and on the saprophytic and bacterial contaminants of pea seed, and on the size and green weight of, and chemical injury to, the sprouts*

PROPRIETARY DUSTS CONTAINING VOLATILE CHEMICALS

Dust applied to seed	Germination	Seeds or sprouts contaminated with—				Length of plumules	Length of radicles	Green weight of sprouts	Chemically injured tests (ratio [2])
		Rhizopus nigricans	*Penicillium spp.*	*Dematium pullulans*	Bacteria				
Ceresan	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Inches*	*Inches*	*Grams*	
Treated	90.4	0.4	0.2	0.1	7.0	2.7	2.7	41.9	2/63
Check	79.7	32.8	1.6	31.4	16.3	2.2	1.8	26.5	0/63
New Semesan Jr									
Treated	85.8	0	1	0	6	-		42.7	0/10
Check	81.9	17.3	2.4	18.5	6.9	-		40.1	0/10
Semesan									
Treated	95.5	0	6.5	0	.5	2.6	2.5	70.5	0/8
Check	90.0	3.2	1.4	11.3	1.5	2.3	2.3	66.1	0/8
PROPRIETARY DUSTS CONTAINING NONVOLATILE CHEMICALS									
Barbak C:									
Treated	94.0	2.2	1.8	0	3.2	......	.	43.0	3/10
Check	90.2	28.2	0.8	25.0	6.0	......	. .	45.5	3/10
Merko:									
Treated	90.8	1.4	.4	0	2.8	1.7	2.7	45.1	1/60
Check	85.7	28.0	3.9	48.0	8.6	1.5	1.6	39.2	0/60
Sanoseed:									
Treated	89.8	5.0	2.0	0	3.7	....	......	40.8	8/40
Check	91.6	14.4	5.4	17.8	6.6	....	....	40.3	0/40

TABLE 2.—*Influence of certain proprietary dusts and mercury compounds on the germination and on the saprophytic and bacterial contaminants of pea seed, and on the size and green weight of, and chemical injury to, the sprouts*—Continued

DILUTIONS OF NONVOLATILE CHEMICALS

Dust applied to seed	Germination	Seeds or sprouts contaminated with—				Length of plumules	Length of radicles	Green weight of sprouts	Chemically injured tests (ratio)
		Rhizopus nigricans	*Penicillium spp.*	*Dematium pullulans*	Bacteria				
Mercuric chloride:[2]	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Inches*	*Inches*	*Grams*	
17 percent[1]	84.3	0	0	0	0	---	--	29.2	1/10
Check	85.5	39.2	3	95.5	4.2	----	-	30.5	0/10
Mercurous chloride:									
Undiluted	92.0	5	6	0	1.6			33.5	6/10
Check	86.8	13.6	3.2	4.8	5.8			36.4	0/10
Phenyl mercury nitrate									
25 percent[1]	93.1	1	1	3	1.5			38.3	7/25
Check	90.9	9.6	6.3	35.4	4.3			39.5	0/25
10 percent[1]	94.4	3	7	0	1.5			49.2	0/12
Check	93.9	9.0	3.8	25.0	4.1			48.6	0/12
5 percent[1]	91.9	.1	3	0	2.1	----	- --	48.2	0/16
Check	92.8	1.2	1.8	9.8	3.6	- - -	- -	47.8	0/16

[1] Diluted with bentonite clay, the chemical is expressed in percent by volume

[2] See footnote 4, table 1.

As shown in table 2, Ceresan effectively reduced fungus contaminations. This dust is outstanding for its uniform control of fungus saprophytes irrespective of the variety or age of the seed, or of the species of fungus. Bacteria were more tolerant of this chemical (ethyl mercury chloride) than were fungi, and complete elimination of bacteria was never observed. Ceresan did, however, localize the bacteria to the dead seeds or points of infestation and usually inhibited their rapid decay. The slimy condition, so objectionable in handling untreated tests, was practically unknown in Ceresan-treated rolls of peas.

Radicles in the Ceresan-treated tests were consistently longer than those in the untreated tests largely because Ceresan prevented fungi and bacteria from destroying them. In several lots of stored seed the average lengths of radicles in the untreated tests were only one-half the length of those in the treated rolls. The differences between radicle lengths of treated and untreated seeds were usually less in freshly harvested than in stored peas of the same varieties. Comparative radicle lengths for 23 uncontaminated samples were 2.3 inches for the untreated, and 2.9 inches for the Ceresan-treated. In tests of stored seed Ceresan often increased the length of radicles by 1.0 inch.

The plumules were never seriously injured by associated saprophytes, and consequently Ceresan effected only slight increases in their lengths.

Comparative green weights of treated and untreated seeds also strongly indicated that Ceresan increased the growth of pea sprouts. In 11 highly contaminated lots the average weight of sprouts from 100 untreated seeds was 26.8 gm., from treated seeds 60.6 gm. Similar weights for 33 noncontaminated lots were 39.9 gm. for the untreated and 44.3 gm. for the treated.

New Semesan Jr. and Semesan were used with a limited number of varieties of pea. They generally decreased the percentage of contaminated seeds and usually increased the percentage of apparently normal seedlings. Gains in weight of sprouts were not so consistently secured by these mercurials as with Ceresan. Uniform dosages were easily obtained by the excess dust-hand-agitation method since both New Semesan Jr. and Semesan adhered well, and the excess dusts were easily removed.

NONVOLATILE PROPRIETARY DUSTS

The only serious disadvantage in the application of the highly effective volatile dusts in the seed laboratory is the danger of inhaling the poisonous gases. For this reason certain nonvolatile dusts were used extensively. When a choice was permissible, the laboratory assistants invariably selected one of the latter materials. The data in table 2 were averaged from at least 10 varieties used as test material for each dust.

From a consideration of germination, fungus elimination, development of sprouts, and lack of injury, Merko was found to be very satisfactory for laboratory work. The other materials were equally suitable in several respects, but for excess dust treatments the percentages of the active chemicals in each dust apparently were too high.

DILUTIONS OF NONVOLATILE PURE CHEMICALS

A dust composed of one part of mercuric chloride and five parts of clay by volume had a high fungicidal-phytocidal ratio. As shown in table 2 highly contaminated peas were rendered entirely free of both fungi and bacteria by treatment with the diluted mercuric chloride dust. Chemical injury was noted in only one test, and that was probably the result of improper mixing of the materials. Later experiments showed that as a laboratory fungicide mercuric chloride compares favorably with New Ceresan. Dusts containing 17 percent of either material consistently eliminated fungi although mercuric chloride seemed not to accelerate growth or to effect increases in green weight. Even when diluted with 10 times its bulk of clay, mercuric chloride decreased both fungi and bacteria to a degree that permitted accurate and rapid counting of normal sprouts. Germinants developing in contact with mercurous chloride may exhibit all the typical characteristics of mercury poisoning. In spite of the low solubility of mercurous chloride in water sufficient amounts of the chemical will adhere to pea seeds to cause marked retardation of growth. When carefully applied to dry seeds and with the excess entirely removed only slight injury was observed. On several occasions the chemical adhering to moist pea seeds completely ruined the rolls of germinants. On the other hand dilution of mercurous chloride with equal volumes of an inert dust entirely prevented phytotoxicity, but contaminants were not eliminated. In case of application, freedom from volatilized gas, and absence of discoloration of working surfaces the mercurous compound compares favorably with mercuric chloride. Since the latter can be diluted with five times its volume of an inexpensive inert material it is preferable to the more expensive mercurous chloride. The two compounds are compared in table 2.

Phenyl mercury nitrate although very insoluble in water proved to be an efficient seed protectant. It was easily compounded with either talc or clay. The optimum dilution varied somewhat with the variety of pea seed and evidence of infestation but a mixture of one part of chemical to nine parts of diluent proved satisfactory in several experiments. Compared to either of the ethyl mercury compounds phenyl mercury nitrate would be exorbitantly expensive even for laboratory disinfestation.

DILUTED AND UNDILUTED CUPROUS OXIDE

The first studies with chemical protectants were undertaken to adapt the commonly used dust for peas, cuprous oxide, to laboratory technique. Many varieties of peas containing varying percentages of saprophytes were treated. Applications of the undiluted chemical by the excess dust method usually resulted in severe injury to the sprouts. Considerably more than the recommended dosage of 2 ounces of dust per bushel of seed actually adhered to the peas. Weight increases of seeds indicated that the 4-ounce rate was often obtained. Since the extremely small amount of chemical required for 100 seeds was difficult to control because of loss on the surface of the treating flask the excess dust method was modified by adding a diluent to the pure cuprous oxide. The per-bushel rate was easily reduced to 2 ounces or as much less as was desired. This method was followed in the experiments the results of which are summarized in table 3.

TABLE 3.—*Influence of cuprous oxide, either undiluted or diluted with clay, on the germination and on the saprophytic and bacterial contaminants of pea seeds, and the size and green weight of and chemical injury to the sprouts*

Type of peas and dust applied	Germination	Seeds or sprouts contaminated with—				Length of plumules	Length of radicles	Green weight of sprouts	Chemically injured tests, ratio[3]
		Rhizopus nigricans	*Penicillium* spp.	*Dematium pullulans*	Bacteria				
Small-seeded:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Inches*	*Inches*	*Grams*	
Cuprous oxide	89.0	0.4	0.8	------	3.9	1.8	1.2	45.0	20/20
Cuprous oxide, clay[1]	88.3	1.5	.9	------	3.1	1.8	1.6	43.7	14/20
Check	90.3	7.5	1.4	------	3.1	2.2	3.1	51.3	0/20
Large-seeded:									
Cuprous oxide	88.7	9.9	1.2	------	7.2	1.1	1.3	41.0	20/30
Cuprous oxide, clay[1]	88.3	14.8	2.3	------	8.8	1.3	1.9	44.8	22/30
Check	87.2	30.3	6.1	------	12.7	1.3	2.3	48.3	0/30
Year-old seed:									
Cuprous oxide	90.5	4.8	.9	0	18.4	1.7	1.1	50.1	75/79
Check	75.4	41.2	2.3	0	29.5	1.9	2.4	44.8	0/79
Canning varieties:									
Cuprous oxide	86.6	.1	0	0	1.4	1.8	1.2	------	8/8
Cuprous oxide, clay[1]	85.5	.5	.6	0	.5	1.8	1.6	------	6/8
Do.[2]	85.3	1.5	.3	0	1.5	2.0	2.4	------	4/8
Check	85.0	3.5	1.7	11.2	1.4	2.2	3.1	------	0/8

[1] Equal volumes of cuprous oxide and bentonite clay.

[2] 1 volume of cuprous oxide to 4 volumes of bentonite clay.

[3] See footnote 4, table 1.

Cuprous oxide was reduced by one or four times its volume of bentonite clay and used in comparison with the unreduced cuprous oxide. The 50-percent cuprous oxide dust gave practical control of molds and bacteria. Seedling injury was less severe in most trials, but in general a decrease in injured radicles was accompanied by a slight increase in molds. The 20-percent cuprous oxide in clay dust further reduced the seedling injury but unfortunately caused a sacrifice in fungus control. Ceresan and New Ceresan usually effected better control than unreduced cuprous oxide and always caused less injury than did 20-percent cuprous oxide.

COPPER STEARATE AND COMBINATIONS OF IT WITH OTHER CHEMICALS

It is difficult to remove cuprous oxide from laboratory benches and tables, and the red spots remaining are objectionable. Furthermore, its phytocidal action overshadows its value as a laboratory fungicide. Copper stearate is preferable in respect to color, adhesiveness to seeds, and ease of removal from working surfaces. Its substitution for cuprous oxide could be justified on these characteristics alone.

TABLE 4.—*Influence of copper stearate used alone and in combination with other chemicals on the germination and fungus contaminants of pea seeds, and on the size of and chemical injury to the sprouts, with comparative data on the use of other seed disinfectants*

Test Nos. and chemical applied as dust	Germination	Fungus contaminated seeds	Length of plumules		Length of radicles		Chemically injured tests ratio [2]
			Average	Maximum	Average	Maximum	
1 to 9:	*Percent*	*Percent*	*Inches*	*Inches*	*Inches*	*Inches*	
Copper stearate	94.9	0.9	1.5	2.3	1.9	3.1	3/9
Copper sulphate	87.6	4.1	1.5	2.2	1.3	2.6	7/9
Cuprous oxide	91.8	1.1	1.5	2.2	8	1.8	9/9
Merko	95.4	2.0	1.8	2.9	2.2	3.6	0/0
Zinc oxide	98.7	2.2	1.3	2.1	1.0	2.2	8/9
Check	29.1	59.5	1.1	2.0	1.4	2.8	0/9
10 to 17:							
Copper stearate	94.4	0	1.6	2.5	1.5	2.7	2/8
Copper sulphate	91.4	0	1.6	2.5	1.2	2.5	5/8
Cuprous oxide	92.5	5	1.4	2.3	8	1.7	6/8
Merko	92.7	0	1.8	2.8	2.4	3.4	1/8
Zinc oxide	90.2	6	1.3	2.2	9	1.8	7/8
Check	87.3	43.3	1.4	2.3	1.2	2.0	0/8
18 to 24:							
Copper carbonate	66.2	16.1	.8	1.3	8	1.6	5/7
Copper stearate	88.8	1.8	1.2	1.9	1.5	2.5	1/7
Copper sulphate	71.8	12.1	1.1	1.9	1.0	1.7	6/7
Copper carbonate-copper stearate [1]	64.2	4.9	1.0	2.0	.7	1.7	7/7
Copper sulphate-copper stearate [1]	34.5	1.2	.5	1.1	5	1.2	7/7
Check	55.2	83.5	.8	1.7	1.0	2.3	0/7

[1] Equal volumes of each chemical.
[2] See footnote 4, table 1.

The data in table 4 show that copper stearate not only is less toxic to pea seedlings than are certain other copper compounds but also it is a better fungicide. This does not accord with various reports of soil tests in which copper stearate appeared to be almost worthless as a seed protectant. The results of the present study, however, indicate that the dosage could be reduced below that of normal adherence without a sacrifice of fungus elimination and seedling protection.

Dusting pea seeds with copper stearate rarely caused an abnormal development of the radicles. A few injured sprouts were found in several tests of seed treated with the undiluted dust. However, the ratio of tests in which sprouts were injured to the total number of tests was never high (tables 4 and 5). Furthermore, the radicles even when injured were not so short and malformed as were those developing in the cuprous oxide-treated rolls. Severe burning and killing of the root tips were not observed.

The plumules from copper stearate-dusted seed were always longer than those from contaminated and untreated peas. In comparison with other copper compounds copper stearate allowed a more normal development of plumules as well as radicles. An actual chemical stimulation probably did not result. Mercury compounds, as represented by Merko in table 4, invariably effected a greater apparent stimulation on either contaminated or fungus-free lots of peas.

TABLE 5.—*Influence of copper stearate dust, copper stearate amended with clay, and New Ceresan, and with both clay and New Ceresan on the germination and on the saprophytic and bacterial contaminants of pea seed, and on the green weight of, and chemical injury to, the sprouts*

Variety of pea seed and chemical applied as dust	Germination	Seeds or sprouts contaminated with—			Green weight of sprouts	Chemically injured tests (ratio [7])
		Rhizopus nigricans	*Dematium pullulans*	Bacteria		
Alderman and World's Record.	*Percent*	*Percent*	*Percent*	*Percent*	*Grams*	
Copper stearate-New Ceresan [1]	65.5	0.5	0	32.3	48.4	11/11
Check	57.5	49.6	11.3	24.1	36.8	0/11
Alaska and Gradus						
Copper stearate-New Ceresan [1]	89.3	2	0	20.4	38.9	19/19
Check	83.8	33.8	27.6	26.3	54.9	0/19
Perfection.						
Copper stearate	94.6	3	8.3	4	43.1	0/20
Copper stearate-clay [2]	95.1	4.8	5.3	6	45.8	0/20
Copper stearate-New Ceresan [3]	94.8	0	0	0	38.3	16/20
Check	94.5	6.5	79.6	1.7	44.1	0/20
Thomas Laxton.						
Copper stearate-New Ceresan [4]	91.4	1	0	1.1	40.2	14/14
Check	85.6	79.5	0	8.9	37.1	0/14
Rogers Chief						
Copper stearate-New Ceresan [4]	95.1	0	0	2	37.8	7/15
Check	93.5	3.8	83.3	1.0	39.0	0/15
Wisconsin Early Sweet						
Copper stearate-New Ceresan [4]	97.0	0	0	2	33.2	4/10
New Ceresan-clay [5]	98.3	2	0	4	33.0	2/10
Check	99.2	11.8	4	1.0	34.3	0/10
Rogers Chief						
Copper stearate-clay-New Ceresan [6]	95.4	0	0	0	40.6	0/18
Check	96.0	0	100.0	0	42.8	0/18
Surprise:						
Copper stearate-clay-New Ceresan [6]	99.0	0	0	.3	35.5	3/10
Check	97.1	16.2	0	8	33.2	0/10
Climax:						
Copper stearate-clay-New Ceresan [6]	93.2	0	0	2.8	23.4	2/15
Check	89.4	17.8	0	7.2	22.0	0/15
Alaska						
Copper stearate-clay-New Ceresan [6]	96.3	0	0	7	30.3	0/15
Check	95.3	2.3	0	1.7	29.0	0/15

[1] A blended mixture of 17 percent of New Ceresan and 83 of copper stearate, by volume.
[2] Equal volumes of copper stearate and bentonite clay.
[3] A blended mixture of 9 percent of New Ceresan and 91 of copper stearate, by volume.
[4] A blended mixture of 5 percent of New Ceresan and 95 of copper stearate, by volume.
[5] A blended mixture of 17 percent of New Ceresan and 83 of bentonite clay by volume
[6] A blended mixture of 80 percent of copper stearate, 16 of bentonite clay and 4 of New Ceresan by volume.
[7] See footnote 4, table 1.

Since monohydrated copper sulphate is toxic to pea seedlings, it was blended with an equal volume of copper stearate in an attempt to reduce seedling injury or at least to effect decreases in fungus contaminations. The seeds were treated by the hand-agitation method, and the excess dust was removed entirely. The mixture appeared to adhere almost as well as did copper stearate. By measuring the exhaustion of material from the treating flasks it was found that the amount of copper sulphate adhering to the seeds was not decreased by the presence of copper stearate. In fact, the greater adhesiveness of the latter entirely compensated for its diluting effect. Naturally, then, as shown in table 4, the toxicity of this mixture exceeded that of either chemical alone, while the percentage of contaminated sprouts was reduced slightly.

A copper carbonate—copper stearate mixture was also compared with the pure chemicals. The seed coverage was slightly greater for the mixture than for copper carbonate alone. The amount of this chemical which adhered to each seed was decreased by the diluent, copper stearate. The germination records indicated that with respect to fungus contaminations and length of plumules and radicles the mixture was inferior to copper stearate alone and only slightly better than copper carbonate.

Copper stearate and New Ceresan were compounded to reduce the toxicity of copper and to prevent the diffusion of volatilized ethyl mercury phosphate. Copper stearate also was diluted with clay by mixing equal bulks of each together.

The first mixtures of copper stearate and New Ceresan compounded for laboratory treatments contained 17 percent of the latter material. The developing pea seedlings were definitely injured, although the apparent germination percentage was often increased. When the seeds were from storage lots the reduction in fungus contaminations due to chemical control usually compensated for the phytocidal effect. The green weight of the sprouts detached from treated seeds was greater than that of the sprouts from untreated peas in a few series of tests, but, as shown in table 5, chemical injury usually caused a marked reduction in green weight.

The 9-percent New Ceresan-91-percent copper stearate mixture also was injurious to the sprouts. When the former component did not exceed 5 percent of the total volume of dust, only a few sprouts appeared to be abnormal. It was necessary, however, to add clay or another diluent to the mixture of New Ceresan and copper stearate to obtain a dust as efficient and safe as diluted New Ceresan alone. The mixture of 80 percent of copper stearate, 16 percent of diluent, and 4 percent of New Ceresan by volume was applied to many large agricultural seeds. Fungus contaminations never exceeded 1.0 percent. The results (table 5) indicated that the copper stearate-clay-New Ceresan mixture is a very efficient, practically nontoxic dust. It is not so disagreeable to use in the laboratory as is a New Ceresan-talc mixture containing 0.83 percent of ethyl mercuric phosphate, is a better fungicide than a copper stearate-clay mixture, and is less toxic to pea seedlings than is undiluted copper stearate. It was tested experimentally on some 30 varieties of peas, with only slight varietal differences.

OTHER COPPER COMPOUNDS

A number of copper compounds, especially those of low solubility in water have caused varying amounts of injury. The influence of a few of these on pea seeds is shown in table 6. The relative value of the materials is indicated by comparison with the New Ceresan and clay mixture. Since the usefulness of a dust depends primarily upon its efficiency in eliminating or localizing saprophytes, it appears that most of the copper compounds cannot be recommended. Good control was secured in some lots of seeds but not in others. By and large, however, several of the more insoluble compounds, the carbonate, hydroxide, and oxychloride in particular reduced molds sufficiently to permit accurate appraisal of the results of the germination tests.

TABLE 6.—*Influence of certain copper dusts on the germination and on the saprophytic and bacterial contaminants of pea seed, and on the green weight of, and chemical injury to, the sprouts*

Variety or type of pea seed and chemical compound applied as dust	Germination	Seeds or sprouts contaminated with—				Green weight of sprouts	Chemically injured tests ratio [3]
		Rhizopus nigricans	*Penicillium* spp	*Dematium pullulans*	Bacteria		
Canning bulks	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Grams*	
Copper carbonate	95.3	1.0	0	0	0	36.6	10/10
Copper hydroxide	95.5	.7	0	0	0	36.6	10/10
Copper oxalate	97.0	5.0	.7	0	.5	41.2	8/10
Copper oxychloride	95.7	.3	.0	0	.3	39.3	8/10
Copper sulphate	94.0	2.0	.7	0	.7	37.7	10/10
Cuprous oxide, clay [1]	95.0	.7	.3	0	.6	38.8	10/10
New Ceresan, clay [2]	97.7	0	0	0	1	40.2	0/10
Check	94.3	11.7	.7	32.3	2.1	41.2	0/10
Commission packets:							
Dusting copper	63.7	59.5	2.5	0	13.2	38.0	9/10
Check	61.5	81.2	2.0	0	23.2	39.5	0/10
Canning peas, 3 varieties:							
Copper oxalate	85.7	15.3	1.3	0	6.7	50.7	8/10
Check	80.0	71.7	1.0	0	17.3	49.3	0/10
Canning peas, 7 varieties.							
Copper phosphate	79.6	74.2	5.2	0	18.0	45.2	0/15
Check	77.1	78.6	3.7	0	19.3	44.3	0/15

[1] A mixture of equal bulks of cuprous oxide and bentonite clay.
[2] A mixture of 1 part of New Ceresan and 5 parts of bentonite clay.
[3] See footnote 4, table 1

ZINC COMPOUNDS AND OTHER CHEMICALS NOT COMMONLY USED AS SEED PROTECTANTS

Various zinc compounds, sulphur, copper-lime-arsenite dusts, and aldehydes in inert diluents were tested on several varieties of peas. In comparison with the copper and mercury compounds the former group was not impressive. Zinc oxide was not a good fungicide even when the dosage applied was injurious to the sprouts. Vasco 4, which contains the oxide and other compounds of zinc was never so effective as the oxide alone, nor did it effect as definite a retardation of radicle elongation. Further reduction of the available zinc by mixing graphite with Vasco 4 resulted in a material with only slight fungicidal properties. Zinc chromate and zinc stearate were superior to zinc oxide chiefly because they did not injure the sprouts. Table 7 presents an accurate comparison of these materials.

Zinc oxide and, to a lesser extent, other zinc compounds were easily adapted to laboratory usage. They adhered readily to every variety of pea seed. In removal of the excess dust continued sieving seemed to have no effect on the seed coverage so that a uniform dosage was obtained in all trials. The laboratory technicians often expressed a preference for zinc oxide or Vasco 4 largely because these materials were not volatile. That they did not discolor the table and germinator surfaces was the important factor in their selection over other nonvolatile protectants.

Sulphur controlled *Rhizopus nigricans* in only a few lots of peas. Mycelium usually developed from dead seeds regardless of the sulphur coating. When this dust was applied in excess of a normal coverage the percentage of contaminated seeds was decreased. Such a procedure could not be generally followed, however, because it was difficult to determine the amount which would be fungicidal but not phytocidal.

TABLE 7.—*Influence of certain fungicides on the germination and on the saprophytic and bacterial contaminants of pea seed, and on the green weight of, and chemical injury to, the sprouts*

ALDEHYDES

Variety or type of pea seed and material applied as dust	Germination	Seeds or sprouts contaminated with—				Green weight of sprouts	Chemically injured tests, (ratio [1])
		Rhizopus nigricans	*Penicillium* spp.	*Dematium pullulans*	Bacteria		
Retail packets, 10 varieties	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Grams*	
Corona Oat Dust	81.6	20.1	2.0	2.6	8.1	49.0	0/20
Check	76.9	21.9	4.1	34.5	10.5	47.7	0/20
Formacide	78.6	32.1	2.9	3.0	14.9	53.5	2/20
Check	73.1	43.9	4.3	7.5	18.0	48.9	0/20
Perfection and Horsford:							
Grainaide	74.3	63.2	1.0	0	15.8	47.3	1/25
Check	71.0	74.8	2.4	18.3	17.0	45.8	0/25
Telephone and Laxtonian							
Smuttox	94.3	5.5	1.0	0	9.1	33.7	1/10
Check	90.6	11.7	3.9	16.8	11.3	36.2	0/10
COPPER-LIME-ARSENITE							
Garden varieties.							
No. 395	72.5	3.4	1.1	0	3.9	45.0	10/10
No. 397	64.5	9.3	.0	0	31.1	42.5	10/10
Check	85.0	48.7	.5	1.0	11.9	56.1	0/10
Commission boxes, 10 varieties:							
No. 395	79.1	1.6	.6	0	3.9	34.0	15/15
Check	91.0	26.6	3.2	1.8	5.2	41.1	0/15
No. 397	50.6	15.3	10.1	0	29.3	31.7	16/16
Check	80.6	44.4	4.1	5.5	14.4	45.5	0/16
SULPHUR							
Canning varieties.							
Dusting sulphur	81.8	18.6	0	0	10.8	36.2	0/10
Check	89.4	22.0	1.0	0	6.2	35.6	0/10
Retail packets, 10 varieties:							
Dusting sulphur	85.0	15.3	1.7	0	8.2	40.9	1/10
Check	81.6	35.8	3.3	0	13.3	40.0	0/10
ZINC COMPOUNDS							
Thomas Laxton:							
Zinc chromate	92.0	17.9	2.0	0	4.0	------	3/12
Check	76.0	48.2	0	0	16.5	------	0/12
Wisconsin Early Sweet and Perfection:							
Zinc oxide	93.3	9.2	.5	6.5	1.9	34.7	16/20
Check	92.2	18.7	1.0	9.0	4.1	37.2	0/20
Garden varieties:							
Zinc stearate	85.7	10.4	3.0	.9	4.7	89.1	2/11
Check	81.8	43.5	9.6	13.3	11.7	88.3	0/11
Perfection and Surprise:							
Vasco 4	88.2	7.2	1.8	27.5	3.6	37.3	0/20
Check	85.8	16.2	3.6	33.8	6.5	37.4	0/20
New-crop seed:							
Vasco 4	96.0	15.0	2.4	50.0	1.9	48.5	3/20
Vasco 4 plus graphite	95.3	29.5	3.8	43.7	2.4	53.3	0/20
Check	94.7	25.0	5.6	51.8	3.6	51.9	0/20

[1] See footnote 4, table 1.

Several volatile materials recommended for control of stinking smut of wheat and oat smuts were included in this work. The dry pea seeds were treated as in former experiments by agitating them in a flask with the dust, followed by a removal of the excess material. The seeds were at once placed on damp towels which were folded into loose rolls. No attempt was made to confine the gases by storing the seeds for a few hours or days as is customary with formaldehyde and methyl aldehyde dusts when employed in smut control. That the folded towels prevented rapid diffusion of the vapors is possible.

The results (table 7) indicate that the black-spot fungus (*Dematium pullulans*) is easily controlled. This is to be expected since it is confined to the surface of the seed coat and can be killed before the formaldehyde gas escapes. Naturally neither the formaldehyde dusts nor Grainaide could protect the clean seeds from contamination by *Rhizopus nigricans* or *Penicillium* spp. The fact that injury to the sprouts has only rarely been observed proves definitely that the vapors diffuse from the towels within a few hours.

The copper-lime-arsenite dusts,[6] valuable as foliage protectants and for control of stinking smut of wheat, were found to be good fungicides, but they severely injured the seedlings. They were used as compounded in Wisconsin for smut control or foliage dusts. A comparison of the results from the two dusts indicated that the toxicity could be reduced by the addition of an inert carrier. The increase in percentage of bacteria-contaminated seeds was attributable entirely to killing of the seeds.

COMPARISON OF SEVERAL DUST PROTECTANTS

A number of ½-pound bulks of pea seed for sale in 1935 germinated erratically in both towel and soil tests at that time. They were held in dry cool storage and again germinated in July 1937. Since seeds were plentiful and accurate percentages of normal sprouts were desired, each sample was treated with Ceresan, 50-percent copper carbonate, copper stearate containing 17 percent by volume of New Ceresan, cuprous oxide, Merko, 17 percent of New Ceresan in talc, Smuttox, and zinc oxide. Although the exact data cannot be presented, it is sufficient to say that with the excess-dust method Ceresan, copper carbonate, Merko, New Ceresan, Smuttox, and zinc oxide provided a covering approximate to that obtained by 2 to 2¼ ounces of dust per bushel of seed. Cuprous oxide did not adhere uniformly, ranging from 2 to more than 4 ounces per bushel and depending on the length of the agitation period and the thoroughness of sieving off the excess material.

Judging by the exhaustion of material from the treating flask, the adherence of the copper stearate-New Ceresan mixture was nearly double that of copper carbonate.

The relative chemical injury occasioned by these materials is shown in table 8. Undeniably the 83-percent copper stearate-17-percent New Ceresan mixture was extremely toxic to peas. As the weight column shows, the sprouts from seeds treated by this material were only three-fifths as heavy as the sprouts from Ceresan-treated seeds and about three-fourths as heavy as the sprouts from copper carbonate-treated peas. Both cuprous oxide and zinc oxide produced somewhat similar results in that the green weights of the sprouts were depressed in comparison with those of the Ceresan-treated tests. Smuttox by the same comparison appeared to have depressed the green weight of the sprouts. That the weight was not greater, however, was owing not to a toxic effect on the sprouts but to imperfect control of the fungus contaminants.

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[6] Dr. G. W. Keitt contributed two dusts of this preparation.

TABLE 8.—*Relative chemical injury occasioned by various chemicals when used as laboratory dusts, and their effect on the germination and fungus contamination of pea seeds, and on the size and green weight of, and chemical injury to, the sprouts*[1]

Chemical applied as a dust	Germination	Fungus-contaminated seeds	Length of plumules	Length of radicles	Green weight of sprouts	Chemical injury	
						Sprouts	Tests
	Percent	*Percent*	*Inches*	*Inches*	*Grams*	*Percent*	*Number*[3]
Ceresan	92.8	0.3	2.5	2.9	47.3	1.3	4
Copper carbonate	87.9	2.0	1.9	1.5	39.2	51.1	36
Copper stearate with New Ceresan[2]	82.8	.3	.8	1.1	29.8	56.3	35
Cuprous oxide	89.9	2.2	1.7	1.2	36.3	65.2	37
Merko	93.1	3.8	1.9	2.8	44.2	1.8	6
New Ceresan	93.4	.2	2.2	2.8	45.4	.9	3
Smuttox	86.6	15.6	2.0	2.3	40.1	.7	3
Zinc oxide	90.7	3.1	1.7	1.4	36.4	58.8	36
Check	80.7	26.8	2.1	2.0	35.8	0	0

[1] All data are averages of 40 individual tests.
[2] A blended mixture of 1 volume of New Ceresan with 5 volumes of copper stearate.
[3] The number of tests in which chemically injured sprouts were observed.

When copper or zinc compounds are injurious the radicles of the seedlings, as explained by Crosier et al. (*5*), suffer relatively more than do the plumules. By using Ceresan rather than the fungus-rotted controls as a basis for comparison, the radicles of seeds in cuprous oxide-treated tests were reduced to two-fifths of the average normal length. Similarly, the plumules in the same tests were reduced to about two-thirds of the plumule length of the Ceresan-treated peas. The copper stearate-New Ceresan dust, however, reduced the lengths of the radicles to two-fifths, and that of the plumules to less than one-third of the respective portions of the Ceresan-treated sprouts. This latter type of seedling abnormality has been observed previously (*3, 13, 16*) in Ceresan- and New Ceresan-treated barley, oats, corn, and rye, and especially in tests of seeds treated with extremely large amounts of either chemical. Undoubtedly the injury in the studies with peas was also caused by excessive dosages of New Ceresan since three-fourths of an ounce of New Ceresan per bushel of seed is nearly the maximum noninjurious dosage for peas, and the actual rate in these experiments was 1 to 1½ ounces per bushel.

Another difference between copper and mercury injury to peas is the type of radicle abnormality. Toxic dosages of New Ceresan caused a shortening and often a thickening of the radicles. Usually the entire seedling appeared to have been decelerated in its growth. Copper-injured radicles were twisted, discolored, extremely brittle, and often without secondary roots. The root tips appeared to have been corroded or burned by a strong mineral acid. The copper injury was apparently permanent, the mercury injury in part was of a temporary nature.

The data in table 8 strikingly illustrate the value of the several mercury compounds in comparison with the other materials studied. New Ceresan when properly diluted with an inert carrier was conspicuously effective against common storage molds. Ceresan and Merko were less perfect fungicides, but the seedlings were normal and usually stronger than were those from the untreated peas. The abnormal seedlings in the copper- and zinc-treated rolls proved fully as troublesome in vitality determinations as did the fungus contaminations in the untreated rolls. Smuttox was not uniformly effective in mold control and never excelled Ceresan nor New Ceresan.

INSTANT DIP TREATMENTS

Although dust treatments appear to be justifiably preferred over dips or soaks for treatment of large bulks of seed, the opposite was true in seed laboratory work. The difficulty of obtaining a uniform dosage probably accounts for occasional injury and nonuniform control of molds in the dust applications. There was also considerable waste of material by the dry method. The diffusing dust particles were both disagreeable to, and dangerous when inhaled by the workers. An attempt was made to determine the correct concentrations of several materials in either suspension or solution in water so that they might be substituted for the dry materials.

TABLE 9.—*Influence of certain proprietary mercury dips on the germination and on the saprophytic and bacterial contaminants of pea seed, and on the size and weight of, and chemical injury to, the sprouts*

BARBAK C

Variety or type of seed and concentration of dip	Germination	Seeds or sprouts contaminated with—				Length of plumules	Length of radicles	Green weight of sprouts	Chemically injured tests ratio [4]
		Rhizopus nigricans	*Penicillium* spp	*Dematium pullulans*	Bacteria				
Stored bulks	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Inches*	*Inches*	*Grams*	
1.25 percent	92.5	2.8	0.8	3.4	4.9	0.7	1.6	41.1	12/15
Check	83.8	48.4	3.2	7.0	7.7	1.1	2.5	41.1	0/15
Retail packets									
1.0 percent	89.2	0.2	0	0	5.7	------	------	31.6	2/23
Check	76.9	43.3	1.9	18.1	15.8	------	------	27.4	0/23
1.0 percent, filtered [1]	80.8	2	0	0	11.7	1.5	3.1	42.2	0/20
Check	77.8	24.7	5.9	0	15.8	1.4	2.9	39.9	0/20
World's Record									
1.5 percent	84.8	1.4	0	0	4.4	------	------	30.8	2/15
Check	74.6	87.8	11.6	2.0	17.4	------	------	27.8	0/15
New-crop Idaho									
1.5 percent, filtered [1]	84.1	[2] 3	0	0	6.1	1.4	3.0	52.6	0/15
Check	84.3	8.1	18.2	17.0	8.0	1.3	2.9	50.7	0/15
World's Record									
2.0 percent	85.4	1.0	.4	0	4.0	------	------	21.8	7/20
Check	76.4	53.0	13.8	0	14.8	------	------	16.0	0/20
New-crop Idaho									
2.0 percent, filtered [1]	89.2	0	0	0	6.8	1.5	2.6	46.8	2/18
Check	86.5	4.3	1.0	2.5	8.2	1.5	2.7	47.9	0/18

MERKO

Variety or type of seed and concentration of dip	Germination	*Rhizopus nigricans*	*Penicillium* spp	*Dematium pullulans*	Bacteria	Length of plumules	Length of radicles	Green weight of sprouts	Chemically injured tests ratio [4]
Retail packets:									
1.5 percent	82.1	8.5	2.3	0	9.1	------	------	------	0/22
Check	80.7	13.3	4.0	0	13.4	------	------	------	0/22
New-crop canning									
2.0 percent	89.5	.7	.2	0	6.6	------	------	------	0/25
Check	89.0	4.4	3.1	0	6.8	------	------	------	0/25
New-crop mixed									
2.5 percent	92.3	2.8	2.5	1.7	1.0	------	------	------	0/20
Check	89.3	8.5	1.8	16.1	1.3	------	------	------	0/20
Garden varieties.									
3.0 percent	83.0	6.7	4.0	5.0	4.8	0.9	2.4	41.0	4/39
Check	82.5	13.7	5.5	24.2	5.0	.8	2.8	38.3	0/39

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[1] Clear solution of ethyl mercury chloride obtained by filtering off the nonsoluble carrier.
[2] Growth of *Ascochyta pisi* inhibited by treatment.
[4] See footnote 4, table 1.

TABLE 9.—*Influence of certain proprietary mercury dips on the germination and on the saprophytic and bacterial contaminants of pea seed, and on the size and weight of, and chemical injury to, the sprouts*—Continued

NEW CERESAN

Variety or type of seed and concentration of dip	Germination	Seeds or sprouts contaminated with—				Length of plumules	Length of radicles	Green weight of sprouts	Chemically injured tests ratio
		Rhizopus nigricans	*Penicillium* spp.	*Dematium pullulans*	Bacteria				
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Inches*	*Inches*	*Grams*	
Commission boxes									
0.15 percent	80.1	1.2	0.1	0	10.7	----	----	39.8	0/32
Check	71.4	38.4	3.2	0	21.8	--	--	35.2	0/32
New-crop, 25 varieties.									
0.18 percent	93.1	2	1	0	2.2		----	27.7	1/65
Check	90.3	5.7	1.5	3.3	3.4	--	----	26.6	0/65
Stored seed:									
0.18 percent, decanted [3]	85.5	2.8	7	0	7.2	----		21.8	0/25
Check	69.5	69.9	25.3	11.3	22.8	---		15.3	0/25
Retail packets									
0.20 percent	80.2	2.0	2	0	14.0	1.0	2.0	49.0	2/19
Check	76.6	34.1	7.5	0	18.5	1.0	1.9	49.6	0/19
Year-old bulks:									
0.20 percent, filtered [3]	88.0	4	1	0	5.3	.7	1.8	47.4	0/20
Check	81.7	35.2	4.5	2.5	15	.7	1.8	47.0	0/20
Commission boxes									
0.25 percent	66.0	3.7	1.3	0	26.1	.7	1.5	44.5	4/33
Check	60.8	63.2	23.0	20.4	37.7	.8	1.4	43.3	0/33
New-crop garden									
0.25 percent filtered.	84.2	0.3	.5	0	10.[illegible]	.7	1.8	47.2	1/10
Check	81.7	2.6	7.0	2.7	12	.7	1.9	46.[illegible]	0/10
Commission boxes									
0.30 percent	54.2	2.4	1.3	0	42.7	.9	1.9	49.6	2/11
Check	44.9	94.3	19.1	0	52.3	.8	1.8	48	0/11
Year-old bulks									
0.30 percent filtered	91.2	1.3	.2	0	2.6	1.1	2.2	53.9	2/12
Check	84.7	38.3	4.4	10.9	9.1	1.0	2.3	55.3	0/12
SANOSEED									
Retail packets.									
1.5 percent	69.9	30.4	2.1	0	13.8	--	-	36.6	0/30
Check	56.2	86.5	5.4	0	28.5	--	--	32.9	0/30
Storage bulks.									
2.0 percent	81.5	4.7	.4	0	8.6	-----	----	34.0	2/16
Check	76.6	55.0	1.5	0	15.0	------	----	34.0	0/16

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[3] Clear solution of ethyl mercury phosphate obtained by decanting or by filtering off the nonsoluble talc carrier.

PROPRIETARY MERCURIALS

Suspensions of New Ceresan were tested extensively at concentrations of 0.15 to 0.30 percent. The results, summarized in table 9, indicate that these concentrations are effective disinfestants and are not toxic to pea sprouts. The 0.18-percent concentration was selected by the State seed-testing laboratory for use in treating peas, beans, and corn. The amount of ethyl mercury phosphate adhering to the seeds was nearly identical with that obtained by the dry method when employing a 17-percent New Ceresan dust.

Hofer and Hamilton (*6*) used an aqueous suspension of only 0.125 percent of New Ceresan for disinfesting legume seeds and reported satisfactory control of fungi. Recent work in the seed-testing laboratory with vetch-seed disinfestation was successful with a 0.30-percent instant dip. Very little injury was noted when the seeds were transferred directly to agar plates, and the percentage of sterile transfers significantly exceeded those from a chlorine solution.

The dip and dust equivalents for New Ceresan having been determined, the correct concentrations for other materials were readily computed. The substitution of dip for dust treatments with other mercury compounds has not, however, always been successful. The mercurial in Ceresan is soluble and has given uniformly good disinfestation, but Merko and Sanoseed (table 9) have not. It is difficult to keep these materials in suspension, and the mercury adhering to each seed cannot be accurately controlled. In laboratory practice it is necessary to pour the material over the seeds several times or to agitate the beaker containing the seeds and the suspension. Based on the effective dosage of New Ceresan, a 1-percent suspension of Ceresan, Merko, or Sanoseed should prevent development of saprophytes, but it did not always do so.

For a time a 0.9-percent Ceresan suspension was regularly used in routine testing of certain vegetable and flower seeds. Profuse growths of *Rhizopus nigricans* were eliminated, although *Penicillium* spp. and bacteria persisted on the dead seeds. Increases in the strength of Ceresan up to 1.5 or even 2.0 percent effected only a slightly better average control but did prevent the occasional development of saprophytes on highly contaminated seed stocks. Apparently this is true for peas as well. Since the application of a 1.5-percent suspension was not accompanied by any definite injury, it is to be preferred over a weaker one.

Barbak C was not studied extensively, but readings from treated and untreated samples of 15 varieties of peas showed satisfactory control of fungi. *Rhizopus nigricans* was easily eliminated, but surprisingly enough *Dematium pullulans* appeared to tolerate a 1.25-percent suspension of this material. The unfiltered 1.25-percent suspension proved toxic to a high percentage of the sprouts. The injured seedlings were very similar to those developing from peas treated with an excess of New Ceresan. That the green weight of the treated tests should equal that of the untreated tests although the phytocidal action was very marked, may appear paradoxical. It should be remembered, however, that the number of sprouts was greater in the Barbak C-treated than in the untreated tests. Furthermore, while growth in the length of both plumules and radicles was retarded by the suspension, the radial growth of the sprouts developing from the treated seeds exceeded that of the untreated sprouts.

FILTERED AND DECANTED SOLUTIONS OF MERCURIALS

In preliminary dip treatments care was taken to agitate the suspensions so that the seeds would obtain a uniform coverage. In later work filtrates or the decanted supernatant liquids were substituted. There was little difference in control of micro-organisms and development of sprouts between the suspension types and filtrate types of Ceresan or New Ceresan. The data in table 9 express the similarity very clearly. Apparently the active medicants, ethyl mercury chloride and ethyl mercury phosphate, were readily soluble in water. Removal of the filtrates from Merko and Sanoseed suspensions depleted their fungicidal value. In several trials the percentages of contaminated seeds were greater in the treated than in the control tests probably because of dissemination of spores in the liquids.

Considerable difficulty was encountered in maintaining a uniform suspension of Barbak C. The inert diluent tended to accumulate on the surface of water and repeated agitation of the sample and water was necessary to obtain a uniform covering. In later experiments the diluent was removed by filtering. The filtrates from 1.0- and 1.25-percent suspensions had no effect on the fungus or bacterial contaminants. Apparently the active ingredient, phenyl mercury cyanamide, was insoluble in water and was filtered off with the diluent.

INORGANIC MERCURY COMPOUNDS

Mercuric chloride has been used for years as a fungicide. Usually the tissue to be surface-sterilized is immersed in a mercuric chloride solution for a definite limited period and is then thoroughly rinsed in water to remove the chemical. This procedure is not practical in routine seed testing. The time element alone is an obvious disadvantage. The period of soaking, unless exceedingly brief, would permit the impoverishment of the mercury content of the solution and necessitate frequent replacements. Removal of the disinfestant after sterilization, moreover, is neither necessary nor desirable since an extended protective action is essential in a crowded spore-laden germinator.

For these reasons an instant-dip method of treating appeared advantageous. Since it had been established that a dilution of mercuric chloride dust affected a control of contaminants almost identical with that obtained by equal dilution of New Ceresan, it appeared likely that similar aqueous concentration would produce comparable control. Thus an 0.18-percent solution of mercuric chloride should be interchangeable with the equivalent suspension of New Ceresan which proved effective in the disinfestation of, and protection to, pea seeds. Laboratory practice has verified this.

A few of the pertinent data of many separate tests are assembled in table 10. Apparently a concentration as low as 0.06 percent is at times an effective fungicide. Sprouts were not appreciably injured until an 0.25-percent concentration was reached. It should be emphasized that all of the results were obtained from instant dips at 70° F., that the seeds were never washed, and that they were placed to germinate immediately. Very probably lower concentrations used as brief soaks would be effective.

In dipping seeds in mercuric chloride solution a small volume, usually 100 or 150 cc., was poured into a glass tumbler and used repeatedly until exhausted. Even when 40 successive lots of 100 seeds each were dipped the concentration of the active chemical apparently was not seriously depleted. Control of fungi was as complete for the last as for the first lot of peas dipped in a 0.15- or 0.18-percent solution. An original concentration of only 0.06 or 0.10 percent might not have given similar results since a slight impoverishment of the solution would have reduced the mercury content to a nonpathogenic strength. Because mercuric chloride is nonvolatile and is also soluble in water it is ideal for laboratory control of saprophytes. In order to compare results with another inorganic mercury, mercurous chloride was used in a few trials. The obvious disadvantage of low solubility in water curtailed extended work with this chemical. Suspensions of 1.0 and 0.5 percent were tested with new stocks of canning peas, giving results as averaged in table 10. The more concentrated suspension compared favorably with other mercury compounds, but the weaker possessed little fungicidal value. No injury was noted in either suspension. A slightly increased concentration could presumably be employed safely and possibly with complete elimination of fungus contaminants.

TABLE 10.—*Influence of solutions of mercuric chloride and suspensions of mercurous chloride on the germination and on the saprophytic and bacterial contaminants of pea seed, and on the green weight of, and chemical injury to, the sprouts*

MERCURIC CHLORIDE

Variety or type of pea seed and concentration of chemical	Germination	Seeds or sprouts contaminated with—				Green weight of sprouts	Chemically injured tests (ratio) [1]
		Rhizopus nigricans	*Penicillium* spp.	*Dematium pullulans*	Bacteria		
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Grams*	
Surprise							
0.06 percent	98.0	0	0.2	0	0.2		0/20
Check	96.8	3.2	.6	1.0	.8		0/20
Canning varieties							
0.10 percent	88.1	4.0	5.1	.1	6.1	48.6	0/15
Check	83.2	50.4	21.8	12.7	13.5	50.5	0/15
Stored bulks							
0.18 percent	87.2	.4	.2	0	7.9	45.7	0/30
0.18 percent New Ceresan	89.0	.1	0	0	6.2	47.2	0/30
Check	81.9	29.2	.9	11.3	11.6	42.4	0/30
New-crop, western							
0.20 percent	95.0	.5	.2	.1	1.8	45.6	4/35
Check	95.2	13.1	.8	20.8	3.8	45.7	0/35
0.25 percent	93.4	.2	.1	0	.7	38.7	11/24
Check	94.3	6.8	.7	62.6	1.8	45.1	0/24
0.30 percent	93.5	.1	0	0	1.3		8/15
Check	93.0	12.1	2.7	49.3	2.3		0/15
Stratagem							
0.40 percent	91.7	.5	0	0	2.8		3/11
Check	90.1	12.2	4.8	1.8	6.7		0/11
MERCUROUS CHLORIDE							
Canning varieties							
0.5 percent	89.9	27.2	1.7	55.5	4.8	50.4	0/10
Check	92.6	29.5	.9	64.0	3.7	49.1	0/10
1.0 percent	94.1	2.2	.8	15.9	1.5	49.2	0/20
Check	93.3	15.9	1.4	27.8	8.5	47.4	0/20

[1] See footnote 4, table 1.

COPPER COMPOUNDS

The majority of the copper compounds recommended for seed protection are not highly soluble in water. Copper sulphate is the most notable exception and should perhaps be regarded as a disinfectant rather than as a protectant. It was first employed in preference to other copper compounds largely because of the ease of dipping seeds in a solution. As may be observed from the data in table 11, the fungicidal-phytocidal ratio of this chemical is extremely low. New Ceresan or mercuric chloride effected significantly better control of fungi and bacteria and without injury to the sprouts. Copper sulphate did not depress the apparent germination of peas but did prevent the normal development of the seedlings. The green weight of the sprouts from the treated seeds rarely equaled that of sprouts in the untreated rolls.

Suspensions of copper oxychloride only slightly reduced the percentages of fungus-contaminated sprouts. The results presented in table 11 indicate that extremely concentrated suspensions would be required to effect significant fungicidal action. Repeated agitation was necessary to maintain a uniform concentration.

TABLE 11.—*Influence of various dip treatments with copper compounds and certain other chemicals on the germination and on the saprophytic contaminants of pea seed, and on the green weight of, and chemical injury to, the sprouts*

Variety or type of pea seed and concentration of chemical	Germination	Seeds or sprouts contaminated with—				Green weight of sprouts	Chemically injured tests, ratio [1]
		Rhizopus nigricans	*Penicillium* spp.	*Dematium pullulans*	Bacteria		
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Grams*	
Stored 1 year:							
1.0-percent copper hydroxide	89.3	29.8	1.0	0	6.4	39.1	0/16
0.18-percent New Ceresan	91.4	1.6	0	0	5.4	40.2	0/16
Check	81.4	71.5	3.3	0	15.6	36.2	0/16
New-crop, garden							
2.0-percent copper hydroxide	90.7	11.6	1.0	12.0	3.3	39.4	8/19
Check	89.4	24.8	1.2	39.0	4.2	43.3	0/19
New-crop, mixed:							
0.5-percent copper oxalate	93.0	6.0	1.5	0	4.0		0/15
1.0-percent copper oxalate	91.5	5.0	2.4	0	4.8		0/15
Check	94.5	8.0	4.0	0	3.2		0/15
New-crop, garden:							
1.0-percent copper oxychloride	83.9	14.6	3.7	2.0	11.3		0/28
0.2-percent New Ceresan	84.8	3	4	.5	5.2		0/28
Check	82.8	22.6	9.7	10.6	10.8		0/28
New-crop, canning:							
1.5-percent copper oxychloride	92.4	4.7	1.2	3.6	8		1/15
0.18-percent New Ceresan	91.7	0	1	0	7		0/15
Check	91.1	5.7	6	44.4	1.1		0/15
Retail packets:							
3.0-percent copper oxychloride	87.5	13.0	7.0	0	8.0		1/10
3.0-percent Sanoseed	88.0	6.5	3.9	0	2.5		0/10
Check	86.5	22.0	11.5	0	8.0		0/10
Canning stocks:							
0.33-percent copper sulphate	96.0	2.0	0	0	1.0		10/20
0.18-percent New Ceresan	96.5	0	0	0	0		0/20
Check	94.4	5.0	1.0	0	1.6		0/20
New-crop, mixed:							
1.0-percent copper sulphate	92.9	11.1	5	4	1.8		21/22
0.15-percent New Ceresan	94.0	1.1	0	0	1.4		0/22
Check	92.7	10.9	2.7	2.4	3.9		0/22
Garden varieties							
2.0-percent cuprous oxide	83.8	6.2	2.0	2.5	5.2	28.5	15/30
Check	90.0	39.5	1.5	41.2	5.0	40.0	30/30
Stored bulks:							
5.0-percent ferrous sulphate	45.0	91.7	30.0	0	42.8	30.5	1/8
Check	69.9	78.6	10.6	0	28.0	45.0	0/8
Stored packets:							
10.0-percent ferrous sulphate	39.8	85.3	45.4	0	57.6	28.8	16/16
Check	76.9	53.9	11.2	0	20.5	40.7	0/16
Canning bulks:							
2.0-percent potassium dichromate	72.8	58.4	6.3	0	23.4	42.9	3/10
Check	73.1	73.5	4.0	16.3	21.6	39.7	0/10

[1] See footnote 4, table 1.

Copper oxalate, copper hydroxide, and cuprous oxide were also studied as dips for several varieties of peas. They remained in suspension for only a few minutes unless disturbed. Usually the seeds were dropped into a beaker containing the aqueous dip and the entire contents were then poured from one beaker to another to obtain a uniform coverage. The results for the individual rolls of peas indicated, however, that the dosages were not identical. Cuprous oxide severely injured the seedlings in a number of the tests while in others the sprouts developed normally. The other copper compounds appeared less injurious but were also less effective in reduction of contaminations.

OTHER CHEMICALS

From time to time a number of chemicals not employed generally as fungicides were tried as laboratory disinfestants or protectants. With practically no exceptions solutions or suspensions of metallic salts either injured the seedlings or failed to control the saprophytes. Potassium iodide seemed promising at first, but the cost of the material curtailed extensive experimentation. Borax and sodium hypochlorite, which have been suggested as fruit washes, proved of little value. The former material did not stay in solution in cold water, while the latter was not effective against molds when used as an instant dip.

Solutions of iron compounds were extremely toxic to the seedlings and actually increased the percentages of contaminated seeds. As shown in table 11 there is no possibility of utilizing ferrous sulphate for protection of contaminated germinants. Potassium dichromate, however, actually increased the green weights of developing sprouts. Solutions of both ferrous sulphate and potassium dichromate discolored the working surfaces as well as the paper rolls.

DISCUSSION

The chemical treating of peas has facilitated the reading of germination tests. The time consumed in applying the chemical has been compensated for by the rapid examinations of the uncontaminated seedlings. The repeating of tests injured by saprophytes has been entirely obviated, resulting in a saving of germinator space and a decrease in the rush periods of seed routine testing. Excessive applications of water, particularly at temperatures above 20° C., encourage fungus and bacterial growth. Untreated samples have often been ruined under these conditions. It has been found that the variations in watering and temperature have little effect on treated peas. Furthermore, when necessary the usual 6-day germination period can safely be prolonged without danger of profuse development of *Rhizopus nigricans*.

In laboratory practice samples of peas were always germinated in duplicate, one treated, and the other untreated. Comparisons of contaminations, strong sprouts, and seedling development were made from the two rolls placed side by side on a tray and therefore were very exact. In severely contaminated samples it was evident which rolls contained untreated seeds. At the end of the germination period *Rhizopus nigricans* entirely obscured the untreated tests while the alternating rolls of treated seeds appeared clean. Mycelium occasionally grew from the untreated into the treated tests. Since only the chemical applied to the seeds was available to protect the seedlings a very severe test of the chemical was created. Under no conditions were peas treated with 0.18-percent New Ceresan, 0.20-percent mercuric chloride, or 1.5-percent Ceresan dip contaminated to any extent. Other mercury compounds were nearly as efficient. The copper and zinc compounds protected the seeds, but the sprouts which developed were often covered by mycelium. Apparently the low rate of solubility of the copper and zinc compounds limited the active zone to the points of application, whereas, the soluble materials, New Ceresan, mercuric chloride, and Ceresan, protected the seedlings as well.

The materials included in this work did not eliminate several fungi. *Alternaria* spp. apparently increased both in prevalence and severity after seed treatment. It seemed certain that in part the visible increase was caused by lack of obscuring growths of *Rhizopus nigricans*. This was very noticeable in several samples dipped in New Ceresan solutions. The percentage of *Alternaria*-infected seeds of certain kinds of flowers appeared to have been doubled after applications of mercuric chloride, cuprous oxide, and New Ceresan. *Rhizoctonia solani* developing from a single infected seed spread throughout and killed entire rolls of peas treated with Ceresan. The growth of *Fusarium* spp. was slightly inhibited, and the infection of adjacent sprouts was prevented by many materials. Jones (*9*) found that *Ascochyta pisi* is tolerant of organic mercurials. In the laboratory, the number of seeds with visible mycelium was reduced from six to one in a sample of Alderman peas and apparently increased in samples of Telephone and of Thomas Laxton which had been treated by New Ceresan. Lack of control was observed also in mercuric chloride-, mercurous chloride-, and Barbak C-treated seeds. Evidently the action of these materials was limited to protection, disinfestation, and very slight disinfection.

Certain seed growers have expressed a preference for sand, soil, or other solid substratum. They contend that contact contamination by micro-organisms is minimized resulting in accurate percentages of germinations. To determine whether evaluations of normal sprouts in towels were approximately similar to soil tests a number of samples were tested in towels and in soil. Neither sand, pasteurized soil, nor muck increased the percentages of the apparent strong sprouts over treated seed in towels. Various chemicals were more instrumental in accentuating percentages of germination.

In any sample of peas containing dead seeds are also seeds which are viable but which easily succumb to the organisms commonly termed "saprophytes." When protected by a chemical or when planted in sterile soil, normal seedlings may develop. If, however, the seeds are contaminated, the chemical functions as a disinfectant and disinfestant as well as a protectant. The original fungus associates are of no moment except insofar as they injure the dry seed. Without the chemical applications, the seeds, whether isolated in soil or on towels, usually do not produce normal sprouts. For this type of seed-stock, testing of treated seeds in towels yields more exact germination data.

While this study was undertaken to determine the value of certain materials as seed disinfestants in laboratory seed-germination practice, it might well serve to anticipate their probable success in field experiments. Questions of stimulations, toxicity, and specific protection from certain organisms could be given a visual approach. Materials which are not injurious to seeds in towel tests could be safely applied to peas planted in any type of soil. That chemicals which caused stunting and weight reduction in towels do not do so in most soils suggests that a portion of the dosage is sterilizing the surrounding soil, or at least, is removed from contact with the seedlings. Much as the glass slide (*15*) was found to be useful in comparing fungicides designed for foliage protection, so can germination trays and towels serve to guide pathologists in the use of seed treatments for the field.

SUMMARY

Germination of pea seeds (*Pisum sativum* L.) in moistened paper towels permits certain seed-borne saprophytes to develop profusely. The pea seedlings may be so severely injured that the seed stocks cannot be appraised accurately.

Since crowded germinator facilities prohibit wide spacing of seeds as a means of isolating saprophytes, various chemicals were studied for inhibiting the growth of seed contaminants.

Seed peas varying from those practically free of any micro-organisms, to others carrying a high contaminant load were treated with both wet and dry applications of different materials. The value of a chemical was based on its inhibiting action to contaminants, phytotoxicity, ease and rapidity of application, health hazards, and cost.

Ethyl mercury phosphate effectively protected both seeds and seedlings from the seed-borne fungi *Rhizopus nigricans*, *Penicillium* spp., *Dematium pullulans*, and to a slight extent from soft-rotting bacteria. New Ceresan inhibited saprophytes even when diluted with 10 times its bulk of an inert material.

Storage of New Ceresan-treated seeds to allow volatilization of ethyl mercury phosphate was not necessary for fungus control.

Other proprietary dusts containing volatile mercurials inhibited growth of contaminants.

The radicles of Ceresan-treated peas were consistently longer than those of untreated seeds. The green weights of the sprouts was also greater for the treated peas.

For laboratory work nonvolatile mercury dusts were preferred to materials containing volatile mercuries, because of the lesser health hazard of the former.

The proper dilution of mercuric chloride with an inert dust compared favorably with New Ceresan in respect to control of contaminants. Mercurous chloride was difficult to apply uniformly to the peas and frequently injured the seedlings. Reduced phenyl mercury nitrate disinfested most of the lots of peas.

Undiluted cuprous oxide severely injured radicles while dilutions which were not phytotoxic failed to control fungi.

Copper stearate rarely induced any abnormality in seedling development. Mixtures of copper stearate with copper carbonate, copper sulphate, or New Ceresan were extremely injurious to pea sprouts.

A dust containing 80 percent of copper stearate, 16 percent of diluent, and 4 percent of New Ceresan completely disinfested 30 varieties of peas.

Zinc chromate and zinc stearate were less injurious than zinc oxide.

Sulphur and volatile aldehydes were not dependable in reducing fungus-seed associations.

Injury to seedlings occasioned by copper compounds was limited to deformation, discoloration, or killing of the radicles. Excessive dosages of mercurials usually caused a deceleration in elongation of both plumules and radicles.

Dip treatments with aqueous suspensions or solutions were safer, more easily controlled, faster to apply, and less wasteful of materials than were dust treatments.

Both mercuric chloride and New Ceresan at concentrations of 0.18 percent consistently eliminated contaminants and usually were effective at lower concentrations.

Dilutions of 0.9 to 1.5 percent of Ceresan controlled fungi with no depressing effect on germination.

Mercurous chloride and the slightly soluble copper compounds did not effect uniform fungus elimination since they were difficult to maintain in suspension.

Certain chemicals used as fruit washes were too evanescent to protect pea seedlings during the 6-day germination period.

Alternaria spp., *Fusarium* spp., and *Rhizoctonia solani* were tolerant of all of the chemicals studied. Elimination of *Rhizopus nigricans* permitted *Alternaria* spp. to develop rapidly and also made its recognition easier.

Ethyl mercury phosphate occasionally decreased the percentage of *Ascochyta pisi*-infected seeds.

The testing of chemically treated peas in towels yielded more exact germination data than untreated seeds widely spaced in pasteurized soil.

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EFFECT OF BORDEAUX MIXTURE AND ITS VARIOUS ELEMENTS ON TRANSPIRATION[1]

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INTRODUCTION

That bordeaux mixture may increase transpiration has long been established, but the recent series of dry summers in the United States has focused the attention of investigators on this effect of bordeaux mixture. Some of the early workers considered that bordeaux mixture decreased transpiration; later it was concluded that it accelerated transpiration. As late as 1931, however, Miller (*11*)[3] concluded that bordeaux gave little or no increase under the conditions of his tests. Obviously bordeaux transpiration [4] is not completely understood.

OBJECT OF THE INVESTIGATION

A study of bordeaux transpiration was made in the greenhouse between December 1935 and June 1936 in connection with an extensive series of experiments on the fungicidal possibilities of several copper compounds, especially red cuprous oxide. Inspection of the data obtained revealed that they were in general agreement with the finding that bordeaux mixture increases the rate of water loss. The data varied from published reports at several points, however.

Speculation upon the correlations and discrepancies in the available data led further and further into the causes of bordeaux transpiration until finally a theory was evolved to account for essentially all of the known facts. The writers' data are sufficient to rough in this theory of bordeaux transpiration, but they are augmented by the published data of other investigators, particularly Wilson and Runnels whose classical set of data on bordeaux transpiration in the publications here cited have facilitated the formulation of this theory.

Several other aspects of bordeaux injury have also been investigated recently at this station. It has been interesting to observe that the various elements in bordeaux bear essentially the same relation to each other whether one studies transpiration, leaf deformation, dwarfing, or defloration [5] (*6*).

MATERIALS AND METHODS

Tomatoes (*Lycopersicum esculentum* Mill.) proved to be good indicator plants for experiments on the recovery of seedlings that

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[1] Received for publication March 31, 1938. Journal Paper No. 261 of the New York State Agricultural Experiment Station.

[2] The writers are grateful to Drs. R. O. Magie and R. F. Suit for helpful assistance in some of this work.

[3] Italic numbers in parentheses refer to Literature Cited, p. 442.

[4] The term "bordeaux transpiration" has been telescoped from the expression "the effect of bordeaux mixture on transpiration of plants." This condensation gains much in simplification, but loses nothing in comprehension.

[5] Horsfall, James G., Hervey, G. E. R., and Suit, R. F. DWARFING OF CUCURBITS SPRAYED WITH BORDEAUX MIXTURE. Jour. Agr. Res. (In press)

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were transplanted immediately after spraying. The variety was a pure-line stock of John Baer. The irregular leaves, however, were unsatisfactory for leaf measurements in experiments where water loss was to be calculated on the basis of leaf area. For such experiments beans (*Phaseolus vulgaris* L.) were used because bean plants had already been used extensively in other transpiration work (*4*). The bean seed was a pure-line stock of Stringless Green Pod Refugee.

Experimental plants were grown in a greenhouse where the air temperature was maintained at 24° C. by a thermostatically regulated steam valve and blower. The temperature control was satisfactory during the night and even on partly cloudy days until April, but the blower in the system proved incapable of handling the heat input on days of brilliant sunshine. In the latter case the temperature rose 5° to 10° above the setting.

For the major part of the work which was conducted with beans, the seeds were sown in pasteurized greenhouse loam in double-walled irrigators previously described (*5*). Irrigators rather than ordinary crocks were used to assure more uniform distribution of water to the roots of the plants. Between tests the soil was removed, the irrigators were treated with 1–100 formalin, and new pasteurized soil was added.

In the first few tests, the bean seedlings were thinned to one in each irrigator, but in later experiments three were left in each pot to reduce plant variability and to give larger leaf area.

As soon as the simple leaves were fully expanded, the surface of the soil was covered with a mixture of paraffin and beeswax (4 parts paraffin and 1 part beeswax) to prevent loss of water by evaporation from the surface of the soil. Small-mouthed bottles were used as reservoirs, one to each irrigator. The soil-moisture system operated with a capillary tension of 17.5 cm of water. The soil thus remained quite moist.

The transpiration losses for the preceding 24 hours were determined by replacing the water taken from the irrigators at 8 a. m., but no readings were taken until the moisture content of the soil had reached equilibrium after the pots were sealed. Readings were taken 2 to 4 days before sprays were applied so that the plants could be arranged into groups of approximately equal transpiration rates.

Most published data on bordeaux transpiration deal with simple differences in water loss between sprayed and nonsprayed plants. In these experiments the unit of leaf area has been taken as a basis for calculating water losses although there are certain difficulties in the calculation.

The difficulties arise because a leaf has two surfaces of equal area which lose water at different rates, and which are affected differently by sprays. When water loss is measured by replacement, the figure represents the sum of the loss from the upper and lower surfaces. For that reason it is common to use the planimeter reading in calculation. Doubling the planimeter reading would give more weight to the effect of the two surfaces, but this would not change the result mathematically and would not be any more accurate because the two surfaces lose water differently.

The data in this paper are expressed on the basis of the area of one side, but this basis must be kept in mind in studying data from tests where the two leaf surfaces were sprayed separately. The area of bean leaves can be calculated from the product of the width and the length by a simple formula already worked out (*4*). Leaves were measured every 4 days. Each measurement was taken as the basis for calculating the amount of water transpired per unit of surface area for the 2 days preceding and the 2 days succeeding the measurement.

Since the time when Duggar and Cooley's work was published (*3*), most data have been calculated as a ratio of sprayed to nonsprayed, the "transpiration ratio" as Martin and Clark (*10*) call it. This method of calculation seems sound when referring two treatments to the same check. It may lead to erroneous conclusions, however. Since the transpiration of nonsprayed plants is the denominator of the fraction used in calculating transpiration ratios, any shift in the size of the denominator will shift the ratio without any alteration in the numerator, which, of course, is the transpiration of the sprayed plants. Therefore, as long as the size of the denominator remains constant as in comparing treatments within one experiment, conclusions based on transpiration ratios are sound. Since the size of the denominator shifts with the weather, transpiration ratios for different weather conditions or for different experiments must be used with caution.

The data on transpiration per square centimeter of leaf area (one side) are given in the tables as transpiration differences (sprayed minus nonsprayed) and as transpiration ratios (sprayed divided by nonsprayed). A correction is applied for any prespray differences in water loss for the various plants.

The basic sprays used were bordeaux 4–4–50 (4 pounds $CuSO_4$, 4 pounds $Ca(OH)_2$, 50 gallons water) and electrolytic red cuprous oxide 1 50, unless otherwise stated. These sprays contain approximately 0.25 percent of copper. Cottonseed oil, lime, and Bancroft clay were sometimes used for comparative purposes. Unless otherwise stated the lime was newly burned in a muffle furnace to assure freedom from carbonation, a factor found to be of considerable importance in this problem. The cottonseed oil was emulsified by shaking it in a flask with 0.5 percent of sodium lauryl sulphate.

Each group of plants was sprayed in the morning, a deVilbiss atomizer being used unless otherwise stated. The irrigators were then distributed over a center greenhouse bench so as to minimize differences in position. No clinostat was available.

RESULTS AND DISCUSSION

In arriving at a general theory of bordeaux transpiration, the various components of the spray mixture have been isolated and studied separately as far as possible. Data are presented also on influence of weather, time, and chemical and physical qualities of bordeaux. The differential effect of bordeaux on the cuticle and the stomata is also discussed. Data on each point are discussed in turn leading up to a final discussion of an hypothesis for bordeaux transpiration.

EFFECT OF WEATHER FACTORS

Under the conditions of these experiments sprays caused greater transpirational increases on beans when they were applied on bright days when the leaves were in direct sunlight than when they were applied on dull days (table 1).

TABLE 1.—*Effect of various sprays on transpiration of beans*

[Water loss expressed as milligrams per square centimeter per day [1]]

EXPERIMENT NO. 4. SPRAYS APPLIED BRIGHT DAY FEB. 6, 1936· BORDEAUX 4-4-50; RED CUPROUS OXIDE 1-50; RED CUPROUS OXIDE 1-50+COTTONSEED OIL ½ PERCENT; LIME 4-50; CLAY 4-50, COTTONSEED OIL ½ PERCENT

Type and date of record	Weather	Water loss for nonsprayed plants	Bordeaux		Red cuprous oxide						Lime						Cottonseed oil		Bancroft clay	
					Alone		Plus clay		Plus oil		Pure		Carbonated		Pure + clay					
			Actual water loss	Transpiration difference [2]	Actual water loss	Transpiration difference [2]	Actual water loss	Transpiration difference [2]	Actual water loss	Transpiration difference [2]	Actual water loss	Transpiration difference [2]	Actual water loss	Transpiration difference [2]	Actual water loss	Transpiration difference [2]	Actual water loss	Transpiration difference [2]	Actual water loss	Transpiration difference [2]
Standardizing period	Fair	110.5	112.9		113.3				119.1				117.8				112.1			
Correction applied			−2.4		−2.8				−8.6				−7.3				−1.6			
Feb. 6	Fair	104.2	194.1	87.5	106.4	−0.6			115.8	3.0			117.2	5.7			115.4	9.6		
Feb. 7	Snow	106.6	148.6	39.6	113.6	4.2			108.1	−7.1			118.7	4.8			105.2	−3.0		
Feb. 8	do	103.0	149.2	43.8	112.2	6.4			103.7	−7.9			113.0	2.7			98.5	−6.1		
Feb. 9	do	90.3	123.2	30.5	105.8	12.7			98.1	−.8			104.4	6.8			90.0	−1.9		
Feb. 10	do	95.9	118.3	20.0	109.6	10.9			92.3	−12.2			107.2	4.0			91.3	−6.2		
Feb. 11	Fair	125.0	147.7	20.3	139.0	11.2			123.9	−9.7			138.2	5.9			118.2	−8.4		
Average		104.2	146.8	40.3	114.4	7.4			107.0	−5.8			116.4	4.9			103.1	−2.7		
Corrected transpiration ratio			138.6		107.1				94.4				104.7				97.4			

EXPERIMENT NO. 6. SPRAYS APPLIED DULL DAY MAR. 9, 1936 BORDEAUX 4-4-50, RED CUPROUS OXIDE+CLAY 1-7-50, LIME 8-50, LIME+CLAY 4-4-50, CLAY 8-50

Standardizing period	Fair	149.0	139.5				148.0				150.0		147.5		137.5				156.0	
Correction applied			9.5				1.0				−1.0		1.5		11.5				−7.0	
Mar. 9	Cloudy	124.0	143.0	28.5			127.0	4.0			133.0	8.0	123.0	0.5	125.0	12.5			118.0	−13.0
Mar. 10	do	153.0	151.0	7.5			158.0	6.0			163.0	9.0	154.0	2.5	149.0	7.5			166.0	6.0
Mar. 11	Rain	69.0	72.0	12.5			73.0	5.0			70.0	.0	68.0	+.5	70.0	12.5			72.0	−4.0
Mar. 12	Snow	51.0	53.0	11.5			63.0	13.0			61.0	9.0	57.0	7.5	57.0	17.5			64.0	6.0
Mar. 13[3]	do	147.0	164.0	26.5			137.0	−9.0			149.0	1.0	122.0	−23.5	140.0	4.5			155.0	1.0
Mar. 14	do	167.0	156.0	−1.5			168.0	2.0			175.0	7.0	151.0	−14.5	162.0	6.5			174.0	0
Mar. 15	Fair	183.0	166.0	−7.5			177.0	−5.0			191.0	7.0	171.0	−10.5	175.0	3.5			194.0	4.0
Average		127.7	129.3	11.1			129.0	2.3			134.6	5.9	120.9	−5.3	125.4	9.2			134.7	.0
Corrected transpiration ratio			108.6				101.1				104.6		95.8		107.2				100.0	

EXPERIMENT NO. 7. SPRAYS APPLIED BRIGHT DAY APR. 4, 1936 BORDEAUX 4-4-50, RED CUPROUS OXIDE 1-50, RED CUPROUS OXIDE+CLAY 1-7-50, LIME 8-50, LIME+CLAY 4-4-50 CLAY 8-50

Standardizing period	Snow	167.3	161.6		170.0		161.0				173.1		171.7		156.7				167.2	
Correction applied			5.7		−2.7		6.3				−5.8		−3.4		10.6				.1	
Apr. 4	Fair	226.9	291.1	69.9	232.0	2.4	240.3	19.7			265.1	32.4	235.8	5.5	244.7	28.4			263.3	36.5
Apr. 5	Cloudy	231.7	254.3	28.3	242.9	8.5	239.1	13.7			267.8	30.3	233.2	−1.9	242.9	21.8			263.2	31.6
Apr. 6	Rain	98.8	116.7	23.6	99.9	−1.6	114.9	22.4			115.1	10.5	103.7	1.5	100.3	12.1			119.4	20.7
Apr. 7	Cloudy	113.8	137.6	29.5	118.6	2.1	128.7	21.2			137.2	17.6	124.2	7.0	121.1	17.9			136.1	22.4
Apr. 8	Fair	192.0	228.6	42.3	194.7	0	201.7	16.0			230.5	32.7	203.1	7.7	199.7	18.3			214.8	22.9
Apr. 9	Cloudy	149.0	180.0	36.7	147.7	−4.0	174.8	32.1			181.4	26.6	165.0	12.6	160.3	21.9			176.1	27.2
Average		168.6	201.4	38.4	172.6	1.2	183.3	20.9			199.5	25.1	177.5	5.4	178.2	20.1			195.5	26.9
Corrected transpiration ratio			122.8		100.8		112.4				114.9		103.3		112.0				118.0	

[1] Water loss read at 8 a. m. Readings given were taken the day after.
[2] Transpiration difference is sprayed minus nonsprayed corrected for differences observed during standardizing period.
[3] Effects of second spray show here.

TABLE 2.—*Effect of spraying upper and lower leaf surface on transpiration of bean leaves*

[Water loss expressed as milligrams per square centimeter per day [1]]

EXPERIMENT NO. 8. SPRAYS APPLIED ON BRIGHT DAY MAY 1, 1936: BORDEAUX 4-4-50; RED CUPROUS OXIDE 1-50; OIL ½ PERCENT

Type and date of record	Weather	Water loss for non-sprayed plants	Bordeaux						Red cuprous oxide						Cottonseed oil			
			Both surfaces		Upper surface		Lower surface		Both surfaces		Upper surface		Lower surface		Upper surface		Lower surface	
			Actual water loss	Transpiration difference [2]	Actual water loss	Transpiration difference [2]	Actual water loss	Transpiration difference [2]	Actual water loss	Transpiration difference [2]	Actual water loss	Transpiration difference [2]	Actual water loss	Transpiration difference [2]	Actual water loss	Transpiration difference [2]	Actual water loss	Transpiration difference [2]
Standardizing period	Cloudy	126.1	134.9		136.7		131.3				129.4		136.4		130.0		129.4	
Correction applied			−8.8		−10.6		−5.2				−3.3		−10.3		−3.9		−3.3	
May 1	Fair	269.7	309.2	30.7	304.8	24.5	291.0	16.1			269.5	−3.5	295.4	15.4	251.4	−22.2	277.3	4.3
May 2	Cloudy	271.0	294.2	14.4	283.2	1.6	281.6	5.4			276.1	1.8	271.9	−9.4	247.0	−27.9	256.4	−17.9
May 3	do	373.3	409.6	27.5	376.1	−7.8	377.0	−1.5			353.4	−23.2	339.8	−43.8	327.0	−50.2	325.5	−51.1
Average		304.7	337.7	24.2	321.3	6.1	316.5	6.7			299.7	−8.3	302.4	−12.6	275.1	−33.4	286.4	−21.7
Corrected transpiration ratio			107.9		102.0		102.2				97.3		95.9		89.0		92.9	
EXPERIMENT NO. 9. SPRAYS APPLIED ON BRIGHT DAY JUNE 2, 1936. BORDEAUX 6-6-50. RED CUPROUS OXIDE 4-50. SODIUM LAURYL SULPHATE SPREADER IN ALL SPRAYS																		
Standardizing period	Cloudy	279.3	280.3		283.2		285.1		265.8		275.8		274.3					
Correction applied			−1.0		−3.9		−5.8		13.5		3.5		5.0					
June 2	Fair	540.6	641.9	100.3	616.6	72.1	539.7	−6.7	442.3	−84.8	498.1	−39.0	461.8	−73.8				
June 3	Cloudy	214.5	286.6	71.1	260.0	41.6	224.9	4.6	194.2	−6.8	210.9	−.1	202.8	−6.7				
June 4	Fair	233.3	288.0	53.7	277.2	40.0	255.2	16.1	213.1	−6.7	231.6	1.8	230.4	2.1				
June 5	do	281.3	312.2	29.9	323.8	38.6	298.1	11.0	261.5	−6.3	281.2	3.4	280.7	4.4				
June 6	do	274.8	288.4	12.6	313.6	34.9	279.9	−.7	262.0	+.7	268.4	−7.1	274.4	4.6				
June 7	Cloudy	194.2	178.0	−17.2	213.4	15.3	194.0	−6.0	195.0	14.3	204.0	13.3	200.9	11.7				
Average		289.8	332.5	41.7	330.2	40.4	298.6	3.0	261.4	−14.9	282.4	−6.1	275.2	9.6				
Corrected transpiration ratio			114.4		112.6		101.0		94.9		98.7		96.7					

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[1] Water loss read at 8 a. m. Readings given were taken the day after.

[2] Transpiration difference is sprayed minus nonsprayed corrected for differences observed during standardizing period

In four experiments the spraying days were bright days and the sun shone directly on the leaves (experiments 4 and 7, table 1, and experiments 8 and 9, table 2). During the 6 days following spraying bordeaux increased the daily average transpiration by 40.3, 38.4, 24.2, and 41.7 mg per square centimeter, respectively. In one experiment (experiment 6, table 1) the spraying day was dull, and bordeaux increased the daily average transpiration rate by only 11.1 mg per square centimeter. It follows, then, that high temperature while the leaves are wet with spray gives a higher bordeaux transpiration than does low temperature under similar conditions.

With reference to the weather that follows spraying, the experiments where sprays were applied on bright days are of interest. For example, in the February experiment (experiment 4, table 1) when cool dull weather followed spraying, the transpiration ratio was 138.6, whereas in the June experiment (experiment 9, table 2) when bright warm weather followed the spraying, the transpiration ratio was 114.4. These findings agree with those of Wilson and Runnels (*19*) who concluded on the basis of transpiration ratios that bordeaux gives a greater acceleration over the checks on dull days than on bright days.

However, it is interesting to examine the same data further by using transpiration differences instead of transpiration ratios. In the February test, the daily average transpiration difference of bordeaux was 40.3 mg per square centimeter, and in the June experiment, it was 41.7 mg per square centimeter. This means that 1 square centimeter of bean leaf surface sprayed with bordeaux lost about 40.0 mg more water daily than a nonsprayed leaf surface—irrespective of the brightness of the day and its effect on total water loss.

It follows that temperature while the spray is being applied seems to be important, but that temperature on succeeding days seems to have no differential effect on bordeaux transpiration.

EFFECT OF TIME

The transpiration differences for bordeaux in the various tests have been plotted against time (fig. 1). The curves show that the transpiration effect increases sharply the first day, and falls just as sharply the second day. In three of the five tests it increased again on the third day, but not so much as on the first day, and again fell off. In one test the secondary rise occurred on the fourth and fifth days followed by a decrease. The third peak in the curve for experiment No. 6 was caused by a second application. In only one test of the five was the secondary rise not observed.

EFFECT OF VARIOUS ELEMENTS IN BORDEAUX MIXTURE

Since bordeaux is a mixture and not a simple compound, its action must be studied by studying the action of its various constituents. Data were accumulated on the effect of the water, the copper, the lime, and a clay. An investigation was then made of the effects of certain qualities of the complete mixture such as hydrogen-ion concentration, filming and coverage, spray load, and capillarity of the film.

Effect of Water

Water is certainly an important element in bordeaux mixture. It is the water that distinguishes bordeaux from copper-lime dust.

It has already been shown that temperature at the time of spraying is important in bordeaux transpiration. The reason is that it is the

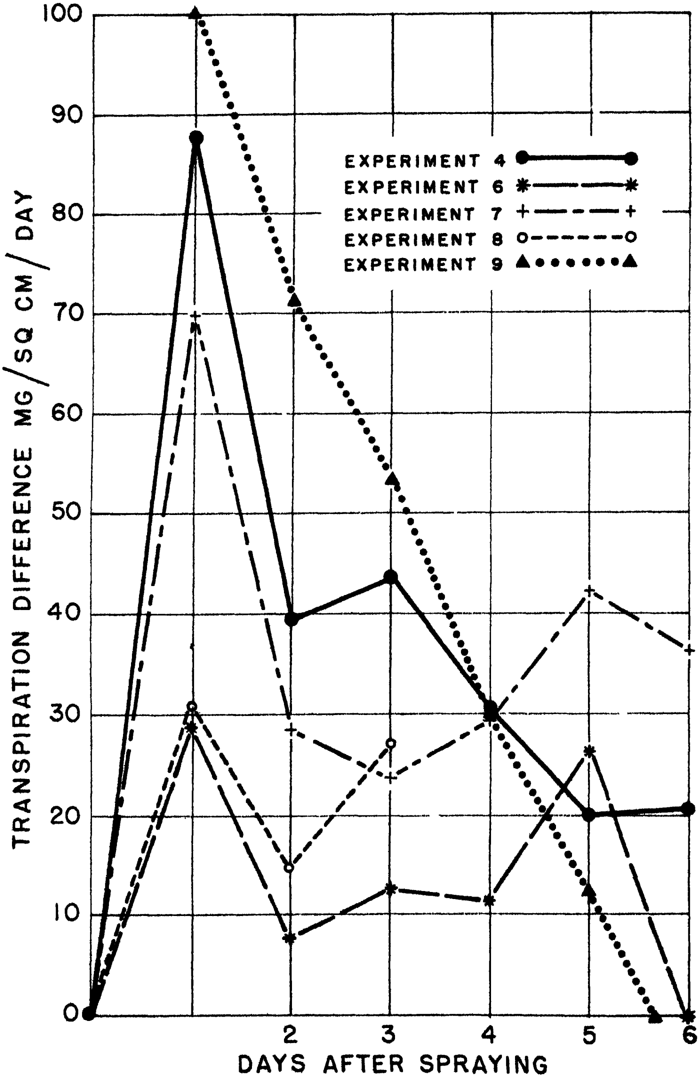

FIGURE 1.—Effect of bordeaux mixture on transpiration of beans on the first to sixth days after spraying. Data taken from tables 1 and 2.

only time (in the greenhouse at least) when water is on the leaf, and when the chemical reactions proceed that open the leaf for the escape of water.

In these tests the temperature on days following spraying was not important. The reason is that no water was present to permit further chemical reactions to proceed.

If water does play a role in producing bordeaux transpiration, dust applied to a dry leaf should produce little or no increase in water loss. Indeed such has been shown to be the case by Wilson and Runnels (*20*) and by Martin (*9*). It has been found at this station [6] that dusts give much less stunting of cucurbits than do sprays of the same materials.

EFFECT OF COPPER

Since the bean and tomato plants used here are not subject to copper flecking, the data obtained were limited to the effect of copper on lime-sensitive plants. The same can be said for essentially all transpiration data on sprays to date.

In the experiments with beans, an insoluble salt, red cuprous oxide, increased transpiration but little or not at all (experiments 4 and 7, table 1, experiment 8, table 2) when used at 1–50, equivalent in copper content to 4–4–50 bordeaux. The same can be said for four times the copper content or 4–50 (experiment 9, table 2). This failure to increase transpiration may possibly be caused by insufficient spray load [7] discussed later.

A soluble copper salt like copper sulphate is, of course, quite destructive to plants. This salt will kill the tissue in leaves of a plant like the tomato that is not considered sensitive to copper in bordeaux. When it kills the tissue it should give a wound acceleration of transpiration, followed by a rapid fall in the rate of transpiration as the tissue dies. Such has been shown to be the case when insufficiently neutralized bordeaux mixture is used (*24*). This reaction probably is not significant in bordeaux transpiration, however, because properly prepared bordeaux mixture contains no free copper sulphate.

EFFECT OF LIME

On the other hand, most bordeauxs do contain some free lime. Bordeaux mixtures without excess of lime can be prepared, but since the system is unbuffered, a small quantity of excess lime makes the system quite alkaline.[8] From the standpoint of using bordeaux in practice an excess-lime formula must be recommended to the farmer since commercial limes may become partially carbonated before use. A study, therefore, of the effect of lime is pertinent to a study of bordeaux transpiration.

Hydrated lime alone increased transpiration markedly although it was not so effective as bordeaux. Likewise, lime showed a devastating effect on tomato transplants. Tomato seedlings were sprayed with various materials and transplanted next day to reasonably moist soil but not watered for 4 days (fig. 2). The plants sprayed with 4–4–50 bordeaux and an equal load of lime (8–50) failed to recover from the transplanting shock. As discussed later the difference between the effect of lime and the effect of bordeaux is complicated by the different filming of the two sprays.

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[6] See footnote 5.

[7] The term "spray load" is used to mean the amount of material retained on the leaf. This, of course, is very difficult to measure. For the purposes of this discussion it is assumed that all sprays are applied and retained in an equal volume in any test. If so, spray load becomes a function of the amount of material per 50 gallons of spray and is so expressed.

[8] See footnote 5.

Carbonation of the hydrated lime reduced the transpiration effect. A lime sample containing 38 percent[9] of calcium carbonate was burned in a muffle furnace. This was then hydrated and compared on the plant with the original carbonated sample (experiments 6 and 7, table 1). When the sprays were applied on a bright day (experiment 7, table 1), the transpiration difference was 25.1 mg per square centimeter per day for the pure lime but only 5.4 mg per square centimeter per day for the carbonated lime. When applied on a dull day (experiment 6, table 1) the pure lime gave a transpiration difference of 5.9 mg per square centimeter per day, whereas the carbonated lime reduced the water loss by 5.3 mg per square centimeter per day. Wilson and Runnels (*18*) report a similar effect of carbonation.

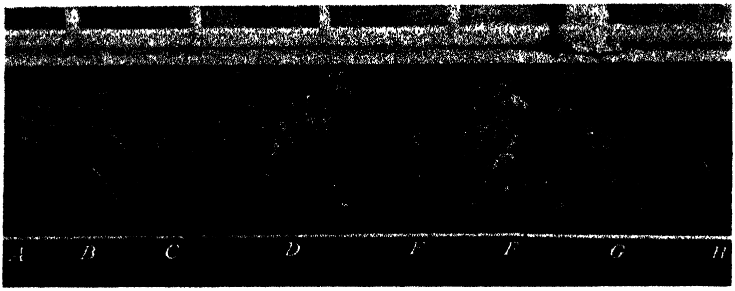

FIGURE 2.—Wilting effect of various sprays applied to tomato seedlings 1 day before transplanting: *A*, Nonsprayed; *B*, bordeaux 4–4–50; *C*, red cuprous oxide 1–50; *D*, bordeaux-cottonseed oil 4–4–½ percent–50; *E*, lime 8–50; *F*, red cuprous oxide-lime 1–4–50; *G*, nonsprayed; *H*, bordeaux 4–4–50.

Since hydrated lime alone caused accelerated transpiration and since reduction of the alkalinity by carbonation reduced the effect, it follows that the alkalinity of bordeaux probably is a significant factor.

There are other data on the effect of alkalinity on bordeaux transpiration. Wilson and Runnels (*20*) found that 4–6–50 copper acetate-lime increased transpiration more than did 1–50 copper acetate minus lime. They attributed this difference to the increased copper content of the former mixture. The presence of lime in the former mixture, however, may well be the cause. Basic copper sulphate (sometimes thought of as bordeaux minus lime) gave them very much smaller water losses than the usual 4–4–50 formula. They (*23*) found also that the addition of lime to liquid lime-sulphur (1–4–50) increased transpiration markedly over liquid lime-sulphur alone (1–50).

EFFECT OF CLAY

Inasmuch as alkalinity was found to be associated with bordeaux transpiration, the effect of an acid clay was investigated. Bancroft clay was the acid material tested (experiments 6 and 7, table 1). This clay, which is sometimes used as a diluent for fungicides and insecticides, is manufactured by United Clay Mines, of Trenton,

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[9] This analysis was made through the courtesy of Prof. A. W. Clark of this station.

N. J. The transpiration rate was increased to about the same degree by Bancroft clay as by hydrated lime. In the first test (experiment 6, table 1) when the spray was applied on a dull day the transpiration differences were 0.0 and 5.9, respectively. In the second test, when the sprays were applied on a bright day, the transpiration differences increased to 26.9 and 25.1, respectively. The addition of red cuprous oxide to the clay had little or no effect on the transpiration difference. Wilson and Runnels (*25*) point out deleterious effects of clays on transplanting losses of tomatoes.

It seems to follow, then, that both acids and alkalis accelerate transpiration of beans.

EFFECT OF HYDROGEN-ION CONCENTRATION

The gross aspects of alkalinity and acidity on transpiration having been observed, it was of interest to relate the subject more clearly to hydrogen-ion concentration. The hydrogen-ion concentration of limewater is approximately 12.3 and that of a Bancroft-clay suspension is about 3.5. These two materials serve to establish the ends of a curve for the effect of hydrogen-ion concentration on transpiration. Since they both accelerate transpiration, it seems clear that a point somewhere between is necessary to establish the low point of the curve. Theoretically the hydrogen-ion concentration of a spray mixture having little or no effect on transpiration should serve to establish the approximate location of this midpoint.

Since red cuprous oxide was known to give little or no acceleration of transpiration, the hydrogen-ion concentration of the suspension of this material was ascertained and was found to be 6.4. The effect of hydrogen-ion concentration of spray suspension on transpiration apparently follows a V-shaped curve with the bottom of the trough somewhere near pH 7.

In studying the stunting effects of bordeaux on cucurbits, Horsfall, Hervey, and Suit [10] have found that the least stunting is obtained with spray mixtures having a hydrogen-ion concentration somewhere near 7.

Wilson and Runnels (*12*; *18*, *pp. 175–179*; *24*) have published data (assembled in table 3) on the effect on transpiration of varying the copper and lime ingredients in bordeaux. Among other effects, varying the copper and lime ratios in bordeaux varies the hydrogen-ion concentration of the mixture The hydrogen-ion concentration of the mixtures they used has been determined. Unfortunately, their mixtures serve to establish only the acid and the alkaline end of the pH curve. They did not make any bordeaux mixtures with neutral hydrogen-ion concentration. Assuming that the transpiration of the nonsprayed leaves was the same as if they had been sprayed with a neutral material as in the present writers' experiments with red cuprous oxide, the curves from these data are also V-shaped. The data in table 3 show some variability of the points on the two ends of the pH scale. The cause of this variability is chiefly variation in spray load as discussed later. In the case of the high copper mixtures, there is also difficulty with soluble copper discussed above.

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[10] See footnote 5.

Table 3.—*Relation of lime and copper variations, spray load, pH value and settling rate on transpiration and desiccation*

[Data assembled from Wilson and Runnels]

Bordeaux formula	pH [1]	Spray load per 50 gallons	Settling [2] rate, height after 1 hour	Transpiration [3] of coleus		Tomato transplants injured [2]	
				October	April	High Ca	High Mg
		Pounds	*Millimeters*	*Grams*	*Grams*	*Percent*	*Percent*
Nonsprayed				70	100	0	0
15-3-50	4.18	18	9.5	61	91	83	80
12-3-50	4.33	15	7.5	73	168	58	52
9-3-50	4.74	12	3.0	77	176	20	32
6-3-50	11.70	9	0.5	85	186	6	0
3-3-50	15.30	6	2.0	95	199	13	7
3-6-50	12.30	9	6.0	101	202	20	13
3-9-50	12.30	12	10.0	95	187	42	22
3-12-50	12.30	15	13.0	91	183	70	62
3-15-50	12.30	18	15.0	86	172	93	87

Bordeaux formula	pH	Spray load per 50 gallons	Desiccation		Transpiration [3]	
			Coleus [3]	Tomato, [4] percent of check	Coleus	Tomato
		Pounds	*Grams*	*Percent*	*Grams*	*Grams*
Nonsprayed			22			128
4-0-50	4.09	4	28	14		
4-½-50	4.36	4½	36	30		
4-1-50	4.36	5	41	41		
4-2-50	11.70	6	45	50		169
4-4-50	12.30	8	50	67		184
4-6-50	12.30	10	51	92		201
4-8-50	12.30	12	49	92		
4-9-50	12.30	13				206
4-12-50	12.30	16	46	63		207
Nonsprayed			25		52	78
0-6-50	12.30	6	29	16		
½-6-50	12.30	6½		22		
1-6-50	12.30	7	32	28		
2-6-50	12.30	8	38	52	106	158
3-6-50	12.30	9	40	60		
4-6-50	12.30	10	47	88	110	162
6-6-50	12.30	12	46	90	102	152
9-6-50	12.30	15	48			
12-6-50	12.30	18	48			

[1] pH-value determined by George Pearce of this station.
[2] Reference (*18, pp. 175-179*).
[3] Reference (*24*).
[4] Reference (*12*).

EFFECT OF FILMING AND COVERAGE

It is recognized immediately that isolating the various chemical components of bordeaux merely gives indications of their functions. Completed bordeaux certainly has physical qualities not possessed by any of the chemical components. The colloidal, gelatinous, filming quality is peculiar.

Lime alone has seldom given quite as much transpiration as the completed bordeaux mixture, even when equivalent copper has been added to it in some so-called insoluble form, and has never given quite as much transpiration when combined with other metallic sulphates (*22*) as when combined with copper sulphate.

It is probable that this is a question of the superior contact which properly prepared bordeaux mixture makes with the leaf. The colloidal bordeaux film obviously makes closer contact with the leaf than do discrete particles of lime. To prepare the best bordeaux films it is commonly suggested to pour the copper sulphate into the lime water rather than the reverse. Such procedure gave Runnels and Wilson (*12*) 70 and 54 percent, respectively, increase in water loss.

The experiment already described showing increased bordeaux transpiration with decreased carbonation of lime is related to filming as well as to increased alkalinity because hydrated lime gives a smoother film than carbonated lime.

Bordeaux loses much of its filming effect upon standing. Wilson and Runnels (*21*) show that 4-day-old bordeaux is much less effective in accelerating transpiration than new bordeaux. The finding (*20*) that soluble salts plus lime give larger transpirational effects than insoluble salts plus lime is probably due to the better filming of the former.

EFFECT OF SPRAY LOAD

In the test on transplants shown in figure 2, 8–50 lime produced more injury than 4–50 lime. This probably is a question of spray load, because the hydrogen-ion concentration of these mixtures is the same. It is true, however, that the 4–50 lime carried also 1 pound of red cuprous oxide, but this probably did not reduce the transpiration. Although the difference is small, Duggar and Cooley (*3*) found more transpiration from 4–6–50 than from 2–3–50 bordeaux, probably because of the difference in spray load.

The experiments of Wilson and Runnels (*12; 18, pp. 175–179; 24*) varying the copper and lime contents of bordeaux mixture are very illuminating from the standpoint of spray load. Obviously as either component is increased while the other remains constant, the spray load also increases. Water loss was measured in three ways: (1) Transpiration from potted plants, (2) desiccation of cut shoots, and (3) injury to transplants. Pertinent data from these authors are arranged in table 3 and as curves in figures 3 and 4. Generally speaking, desiccation and injury to transplants increased with spray load irrespective of whether it was shifted by varying the copper or the lime. Transpiration, on the other hand, increased with the spray load only to a point of about 2 parts of lime to 1 of copper and then decreased sharply.

Possible reasons for the discrepancy between desiccation and transpiration experiments are given in the section on the effect of leaf surface.

It follows that spray load on the leaf is closely correlated with water loss and should be considered in experiments where bordeaux formulas are varied.

EFFECT OF CAPILLARITY OF BORDEAUX FILM

The assumption (*2*) that bordeaux films dissipate water by something akin to capillarity over the large surface of spray film exposed fits the data and helps to explain the effect of filming and spray load.

EFFECT OF OIL

Oil emulsions are known to facilitate filming and coverage of sprays, but it is common knowledge (*17*) that oils generally decrease rather than increase the transpiration rate of plants whether sprayed with bordeaux mixture or not.

Cottonseed-oil emulsion was used as a spreading and coverage agent (experiment 4, table 1; and experiment 8, table 2, fig. 2).

This material alone or in combination with red cuprous oxide reduced the transpiration rate below that of nonsprayed leaves. On first thought this result may be taken as evidence that the oil film bridges over the stomata and curtails water loss from them. On the other hand there is evidence that cottonseed oil does not materially affect stomatal transpiration. In one experiment (experiment 8, table 2) bean leaves sprayed on the lower side (where most of the stomata are) lost more water, if anything, than did bean leaves sprayed on the upper side, although both lost less than the nonsprayed leaves.

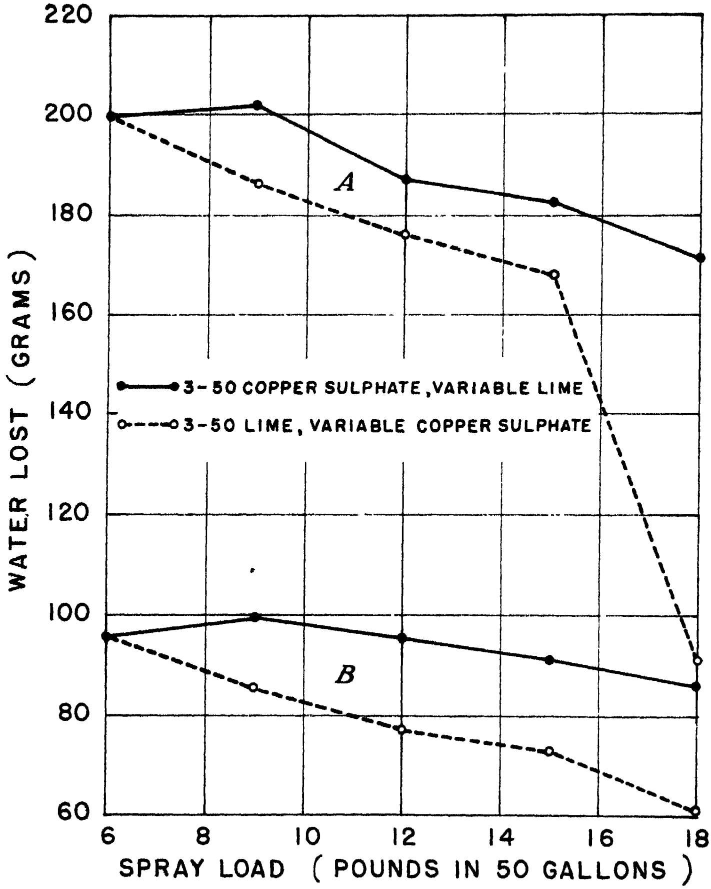

FIGURE 3.—Effect of spray load of bordeaux mixture on transpiration of coleus; data from table 3: *A*, Transpiration in April; *B*, transpiration in October.

Two possibilities for explaining the action of the oil alone in reducing transpiration are (1) that it seals abrasions in the cuticle, and (2) that it functions to make a thicker oily barrier to water loss than the cuticle alone provides.

The reduction in transpiration caused by adding oil to bordeaux may be due to a saturation of the film with oil so that water cannot dissipate itself so readily by capillarity. This effect would be in addition to the basic effect of the oil in the absence of bordeaux.

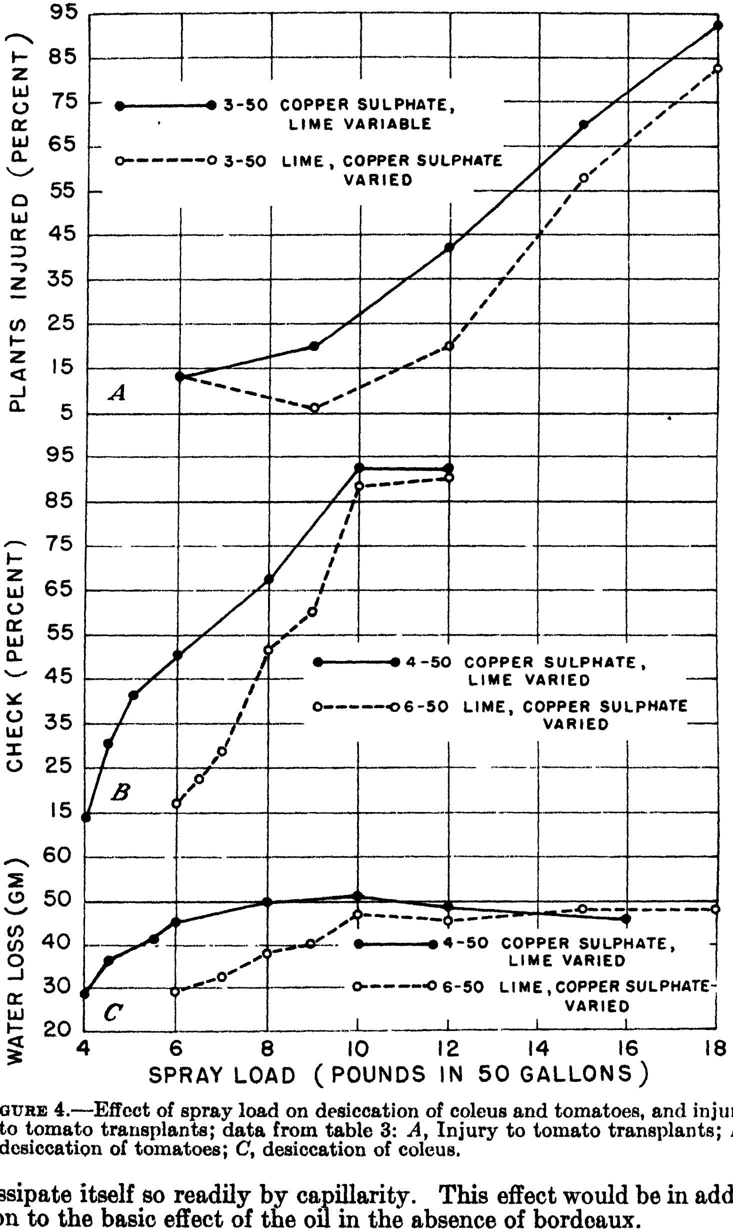

FIGURE 4.—Effect of spray load on desiccation of coleus and tomatoes, and injury to tomato transplants; data from table 3: *A*, Injury to tomato transplants; *B*, desiccation of tomatoes; *C*, desiccation of coleus.

EFFECT OF LEAF SURFACE

Normal leaves give off water primarily through the stomata, although there appears to be some loss directly through the cuticle. In studying bordeaux transpiration, the question arises as to whether only one or both systems are affected and to what extent. Bordeaux might affect transpiration in four possible ways: (1) Increase it by propping the stomata open (*2*) and permitting water to escape, (2) decrease it by clogging the stomata as suggested by Clinton (*1*), (3) increase it by lowering cuticular resistance to water loss, and (4) decrease it by cooling the leaf as shown by Tilford and May (*15*).

EFFECT ON THE STOMATA

The opening or closure effects of bordeaux on stomata have been studied by spraying bean plants on the upper leaf surface where stomata are practically absent, and separately on the lower leaf surface where they are present. If sprays do not affect the stomata, transpiration should be the same whether the material is applied to the upper or to the lower leaf surface. If the spray props the stomata open, the transpiration of leaves sprayed below should be greater than that of leaves sprayed above, because the stomata would then remain open at night and lose water when they would normally be closed. If, however, the spray clogs the stomata, the transpiration of leaves sprayed below should be less than that of leaves sprayed above, because the stomata would lose less water than normal in the daytime.

The latter proved to be the case. In two tests (experiments 8 and 9, table 2) leaves sprayed below with bordeaux transpired less than those sprayed above. Apparently the spray clogged the stomata and did not prop them open. The magnitude of the plugging effect can be calculated with some degree of accuracy. The transpiration difference cancels out the normal effects of stomata and cuticle on nonsprayed leaves. Let the transpiration difference of leaves sprayed above be the cuticular effect of bordeaux and of those sprayed below be the resultant of the effect on lower cuticle and plugging effect on stomata. In the first test (experiment 8, table 2) the transpiration difference on the first day after spraying the upper surface was 24.5 mg per square centimeter per day. The sprayed lower surface should have transpired as much as the upper if no stomatal action were involved, but actually the transpiration difference was only 16.1 mg per square centimeter per day. The 34 percent smaller transpiration of the lower surface than the upper indicates the degree of stomatal plugging. The stomata transpired that much less than normal.

In the second test (experiment 9, table 2) the difference was still larger than in the first test perhaps because of better coverage. In this test the sprayed upper surface transpired 72.1 mg per square centimeter per day more than the nonsprayed on the first day after spraying, while the sprayed lower surface transpired 6.7 mg per square centimeter less than the nonsprayed. In this test the spray on the lower surface plugged enough stomata to eliminate the accelerating effect of the spray on the cuticle.

The magnitude of the stomatal plugging with bordeaux spray can be calculated also from some data of Wilson and Runnels (*16*) who used coleus, which, like the bean, has most of the stomata below. At night when the stomata were closed, the nonsprayed leaves lost 1.09 g of water per hour. Leaves sprayed above lost 2.68 g per hour, and those sprayed below lost 4.66 g per hour. In the daytime, on the other hand, between 10 a. m. and 1 p. m., when stomata are open widest, the nonsprayed leaves lost 17.7 g per hour, those sprayed above lost 17.9 g per hour, and those sprayed below lost 16.5 g per hour.

The magnitude of the plugging effect can be calculated as follows: Let normal cuticular transpiration be the water loss of the nonsprayed leaves at night (1.09 g per hour). Let the normal stomatal transpiration be the water loss of the nonsprayed leaves in the daytime less the normal cuticular transpiration ($17.70-1.09=16.61$ g per hour). Let the bordeaux effect on the lower cuticle be the water loss of the lower-sprayed leaves at night minus the normal cuticular transpiration ($4.66-1.09=3.57$ g per hour).

If the bordeaux sprayed below has no effect on stomata, the daytime theoretical transpiration of the lower sprayed leaves should be the normal stomatal transpiration plus the bordeaux effect ($16.61+3.57=20.18$ g per hour). The actual transpiration of the lower sprayed leaves, however, was 15.41 g per hour ($16.50-1.09$). This gives a reduction from the theoretical of 4.77 g per hour, or 23.6 percent, which represents for this test the magnitude of the effect of stomatal plugging.

EFFECT ON CUTICULAR RESISTANCE TO WATER MOVEMENT

Since the accelerating effect of bordeaux cannot be due to an effect on stomata, it must be an effect on the cuticle, as first proposed by Wilson and Runnels (*16*). The other facts that point to the cuticle are (1) bordeaux increases transpiration at night when stomata are closed, and (2) a spray on the upper leaf surface where stomata are scarce increases transpiration.

It follows, then, that bordeaux mixture has a two-way action on transpiration—it accelerates cuticular transpiration and curtails stomatal transpiration.

The discrepancy in results between desiccation of cut shoots and transpiration of potted plants, discussed above, as regards spray load is interesting from the standpoint of cuticular versus stomatal transpiration. In experiments on desiccation of detached foliage the stomata probably become closed on account of wilting at the very outset of the test. In such a case only the cuticular transpiration is measured. If so, the water loss should increase as it does increase with the spray load because of (1) more complete coverage and (2) increased capillary surface exposed to evaporation by the spray films.

Where total transpiration is measured on potted plants, however, the record represents a summation of cuticular and stomatal transpiration, both of which proceed together. Presumably, increasing the spray load should increase cuticular transpiration both on cut shoots and on potted plants. Increasing the spray load should give decreasing stomatal transpiration, however, so that in the case of potted plants the measurable transpiration should be a resultant of the operation of these two factors. Since the transpiration curve for potted plants generally goes down as the spray load goes up, it is probable that the reduction in stomatal transpiration with increasing spray load proceeds more rapidly than the increase in cuticular transpiration.

Bordeaux should produce less effect on thick than on thin cuticle. That is probably why in the tests bordeaux applied to the lower leaf surface caused more wilting than when applied to the upper, and why (*16*) lower sprayed leaves lost more water at night than upper sprayed leaves (4.66 as compared with 2.68 g per hour). Generally the upper leaf surface in the tests recovered faster than the lower leaf surface (table 2) from the initial transpiration peak, presumably because the thick upper cuticle repaired itself faster than did the thin lower cuticle. If the lower cuticle is more subject to bordeaux damage than the upper, the stomatal plugging effects discussed above are really larger than calculated, because the calculations are based on the assumption that the cuticular effect is the same on both surfaces.

Practically all research to date on bordeaux transpiration has been done in the greenhouse in the North in the wintertime when light intensity is low and where presumably cuticles would be thin. It seems significant that the careful work of Miller (*11*) during 4 years on large tomato vines growing in the open under relatively dry conditions of Kansas showed no significant acceleration of bordeaux transpiration. Miller was probably dealing with a thicker cuticle than that usually found in greenhouses in winter.

On the basis of these findings, it is probable that bordeaux at times may actually reduce transpiration. Such an effect could be produced (1) when cuticular loss is low because of thickened or hardened cuticle and (2) when stomatal plugging is paramount.

EFFECT ON LEAF TEMPERATURE

Wilson and Runnels (*16*) found the sprayed upper surface of leaves also lost less water than the nonsprayed leaves in the middle of the day, when on the basis of the nighttime results they should have lost more. Wilson and Runnels ascribe this to a cooling effect of the spray film on the upper leaf surface. In the case of Miller's experiments, if spray below clogs the stomata and spray above cools the leaf, the total of the two effects may have equaled the cuticular loss obtained.

SUGGESTED HYPOTHESIS FOR BORDEAUX TRANSPIRATION

It seems superfluous to conclude that bordeaux may increase transpiration. The question is rather how does bordeaux increase transpiration. An exit must first be provided for the water. The usual channels, the stomata, seem ruled out (*7*). They appear to be plugged, if anything. The cuticle must, therefore, be opened for the passage of water outward, or perhaps more accurately, its resistance to the passage of water outward must be decreased.

It seems reasonably clear that the lime factor, or rather the alkalinity of the lime factor, is very important if not chiefly responsible for the bordeaux water loss, although acid sprays also seem to increase transpiration, whereas neutral sprays do not. Miller (*11*) says that two of the characteristics of plant cuticle are that it is readily soluble in warm alkalis and readily oxidized by certain acids. The spray material (note larger transpiration from sprays than from dusts) supplies the liquid. A high temperature at the time of spraying provides the warmth and the lime supplies the alkali (in bordeaux). Once the cuticle has been partially saponified by the alkali, it is probable that water can pass outward more readily than before. The bordeaux effect rapidly diminishes (fig. 1). The lime probably carbonates, as shown by Streeter, Mader, and Kokoski (*14*), and is no longer sufficiently alkaline; in any case the spray has dried, so that further saponification cannot proceed. New fats move up from the inner leaf and gradually repair the broken cuticle in 3 or 4 days so that the transpiration effect dwindles.

The acid sprays probably act in much the same manner except that the acids oxidize instead of saponify the cuticle. In either case the resistance of the cuticle to the passage of water is diminished.

It does not seem so certain now that bordeaux will always increase transpiration or that the experiments of Miller (*11*) and Lutman (*8*) can be disregarded. These men worked with mature plants in the field where the plant cuticle is usually thick and hard. The accelerated transpiration effects have been observed chiefly in the greenhouse where the cuticle is usually thin and soft.

It is well within the bounds of reason that if the cuticle is so hard as to resist saponification by alkaline sprays, the lessening effects of bordeaux may overshadow the accelerating ones, so that the summation measured might show even a reduced, not an accelerated, effect.

SUMMARY AND CONCLUSIONS

The experimental work on the effect of bordeaux mixture on transpiration of tomato and bean plants was done in the greenhouse in the wintertime.

Transpiration was measured with autoirrigators and expressed on unit-area basis. The effect of sprays is expressed as transpiration differences or as transpiration ratios.

On the basis of this research and facts available in the literature the following conclusions are reached:

High temperature on spray date favored bordeaux transpiration. High temperature after spray date had little effect.

Of the elements in bordeaux, the lime or rather the lime-induced alkalinity seemed to be the most important, although unneutralized copper sulphate is certainly quite destructive to the leaves.

Any method of making the copper insoluble, even by using it as a dry dust, almost eliminated any of its transpirational effects.

Bancroft clay, a so-called inert material, had an accelerating effect on transpiration presumably because it was acid.

Increase in coverage or filming qualities of bordeaux increased transpiration effects.

Increasing spray load accelerated desiccation of cut plants. On potted plants an increase in spray load increased transpiration to a point beyond which a further increase in spray load decreased transpiration.

Cottonseed-oil emulsion reduced transpiration effects.

Bordeaux decreased cuticular resistance to water loss.

Bordeaux may clog stomata, rather than prop them open, and may increase water loss by capillarity over the surface of the spray film.

A tentative hypothesis of bordeaux-induced transpiration is this: The alkalinity of bordeaux saponifies the cuticle and decreases its resistance to water loss. At the same time, however, it may clog some stomata; it may cool the leaf in bright weather. The balance between these various factors will determine the magnitude of bordeaux transpiration. In the field, where cuticles are thick and hard, it may be true at times that the effect on the cuticle may be so slight as to be overbalanced by the factors for reduced water loss. This point needs further research.

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MORPHOLOGY OF TWO AMERICAN WILD SPECIES OF COTTON AND OF THEIR HYBRID[1]

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INTRODUCTION

Collections of living plants of numerous species of *Gossypium* are maintained by the Bureau of Plant Industry at Riverside and Palm Springs, Calif. Cytological study of these plants by J. M. Webber (*10*),[2] supporting conclusions reached by other investigators, has established the facts that, in all cultivated Asiatic forms and in all species in different parts of the world that have lintless seeds and are unquestionably indigenous in the localities where they occur, the haploid chromosome number is 13, and that, on the other hand, the cultivated American species and the wild or semiwild forms that are evidently related to them and have long lint hairs on the seeds have a haploid chromosome number of 26.[3]

Hybrids between many pairs of species of all three groups (wild lintless, cultivated Asiatic, and cultivated American cottons) have been made by J. M. Webber (*11*), but very few of these have proved fertile. The most outstanding instance of fertility is *Gossypium armourianum* × *G. thurberi*, a hybrid between two wild lintless North American species. F_2 populations, totaling 64 individuals, were grown in 1937 and 1938.

It is hoped that study of interspecies hybrids in the genus *Gossypium* will lead to a better understanding of relationships in this genus and eventually to a more satisfactory classification of the species, and that the puzzling question of the origin of the tetraploid cultivated American cottons may be answered. Realization of this hope seems to depend upon the possibility of inducing doubling of the chromosomes in some of these interspecies hybrids. There is also the possibility, remote, perhaps, but not to be ignored, that cottons superior in resistance to drought, diseases, and insect pests may be developed, if it should prove practicable to obtain fertile hybrids between wild species and some of the cultivated cottons.

The importance of interspecific hybridization in this genus makes it desirable to compare in some detail the morphological characters of *Gossypium armourianum* × *G. thurberi*, the first decidedly fertile hybrid between wild species, with those of the parental forms.

The natural ranges of the parent species do not overlap. *Gossypium armourianum* Kearney (*7, 8*) is known only from the island of San Marcos, in the Gulf of California (lat. 27° 15′ N.). *G. thurberi* Todaro (*Thurberia thespesioides* A. Gray) (*8*) occurs in the mountains of

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[1] Received for publication November 7, 1938. The gross morphology was studied by T. H. Kearney, assisted by J. M. Webber in the classification of the F_2 population. The leaf anatomy was investigated by Irma E. Webber.

[2] Italic numbers in parentheses refer to Literature Cited, p. 459.

[3] Examples are *Gossypium tomentosum* Nutt., *G. taitense* Parl., and *G. darwinii* Watt. These species, which grow in the wild on islands in the Pacific Ocean, have well-developed though short lint hairs on the seeds and are clearly related to the American cultivated cottons.

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southern Arizona and southward in Mexico to the State of Jalisco. The two species are so strikingly different in appearance and structure that fertility in the hybrid between them would scarcely have been expected, although the experience of hybridizers in other groups of plants has shown that morphological similarity is not necessarily an indication of cross-compatibility, and vice versa. Notwithstanding the great morphological dissimilarity of these species, J. M. Webber (*11*) found them to be very similar in cytological behavior.

GROSS MORPHOLOGY OF THE PARENT SPECIES AND F_1

Such of the contrasting characters of *Gossypium armourianum* and *G. thurberi* as were used in classifying the F_2 population and the expression of these characters in the first generation of the hybrid are stated in table 1, columns 1 to 4. The general appearance of the plants and details of some of the characters are shown in figures 1 to 5. Attention is directed particularly to the contrast between the small, round, entire leaf of *G. armourianum* (fig. 3, *A*) and the large, pedately lobed leaf of *G. thurberi* (fig. 3, *B*), which resembles that of the okra-leaf variation in upland cottons.

FIGURE 1.—Plant of *Gossypium armourianum* grown at Bard, Calif., from seed from San Marcos Island, Baja California.

The hybrid (F_1) resembled *Gossypium armourianum* in its thick, somewhat shiny leaf blades, caducous bractlets (falling off long before anthesis), dentate calyx, white stigmas and brown oil glands of the pistil; also (characters not stated in table 1) in its glabrous leaf blades, very small extrafloral nectary on the midvein, relatively long pistil, and number of ovules per locule of the ovary. It resembled *G. thurberi* in the presence of large extrafloral nectaries on the peduncle and in the degree of lobation of the leaf blades; also (characters not stated in table 1) in the color of the older bark, whitish-margined leaf blades, and narrowed apex of the basal sinus of the blade. In the following characters the hybrid was more or less intermediate: Shape of cross section of the twigs; character of the apex and length of the leaf blades; constriction of the midlobe at base; and length of the bractlets.

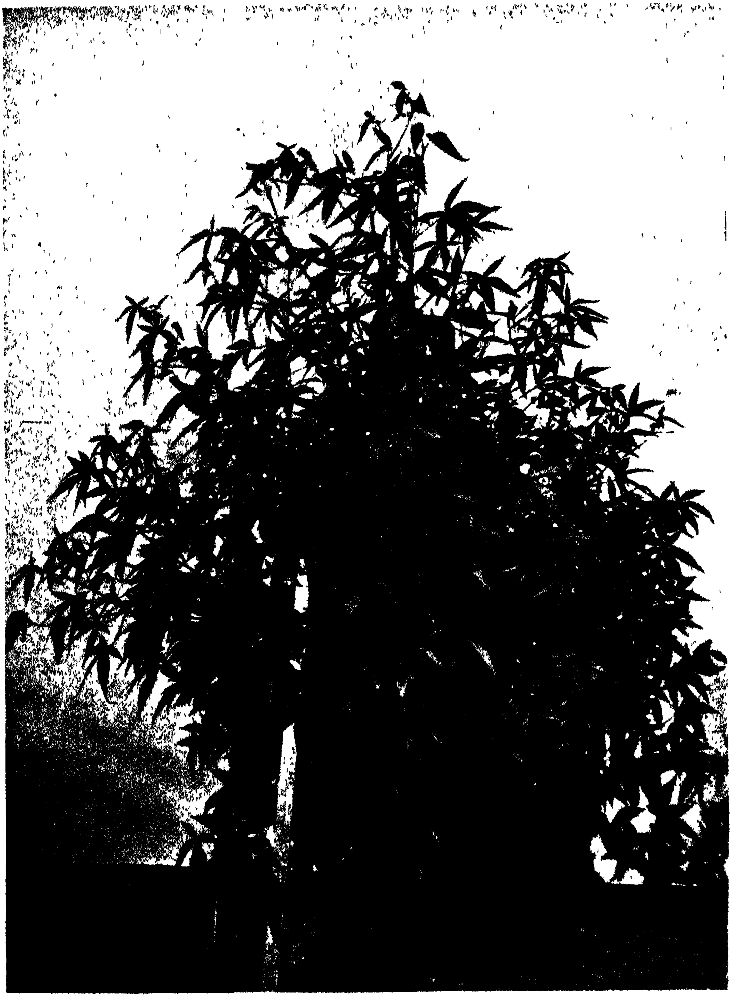


FIGURE 2.—Plant of *Gossypium thurberi* (*Thurberia thespesioides*), grown at Riverside, Calif., from seed from Arizona.

SEGREGATION OF CHARACTERS IN F_2

The classification of the F_2 population in respect to several of the characters that differentiate the parents is shown in table 1, columns 5, 6, and 7. The progenies of the two F_1 individuals (W8 and W9) are treated as one array in column 5, but the two progenies are also classified separately in columns 6 and 7, because they segregated differently in some of the characters. It will be noted that the total number of individuals is smaller in the classifications of flower characters than of stem and leaf characters. This is because some of these F_2 plants failed to produce flowers.

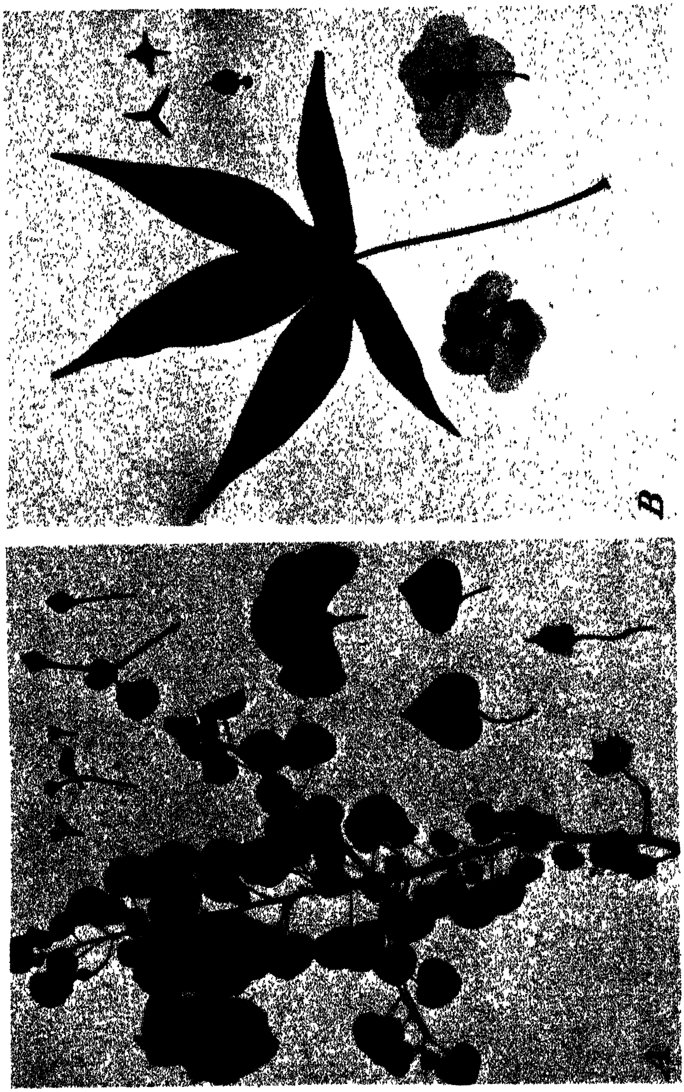


FIGURE 3.—Details of leaves and flowers of parent species, photographed from herbarium specimens, ⅓ natural size. *A*, *Gossypium armourianum*. Note the small, entire leaves and the very small bractlets that persist only while the flower bud is very small (three buds, upper center), dropping off while the bud is still small and long before the flower opens (two buds, upper right). *B*, *G. thurberi*. Note the relatively large, deeply lobed (pedate) leaf and the larger bractlets (upper right) which persist until the boll matures.

The frequency distributions show no evidence of monohybrid segregation except in shape of the cross section of the twigs, in which character there was a near approach to a 1:2:1 ratio, χ^2 of the departure (both progenies as one array) being 0.207. This small value for χ^2 indicates a high degree of probability that the departure of the observed from the expected ratio was fortuitous. On the other hand, the extreme rarity of monohybrid segregation in interspecific hybrids causes one to wonder if the classification was faulty. It should be stated, however, that every effort was made to have it objective and it was not suspected, when the plants were being classified, that definite segregation would appear.

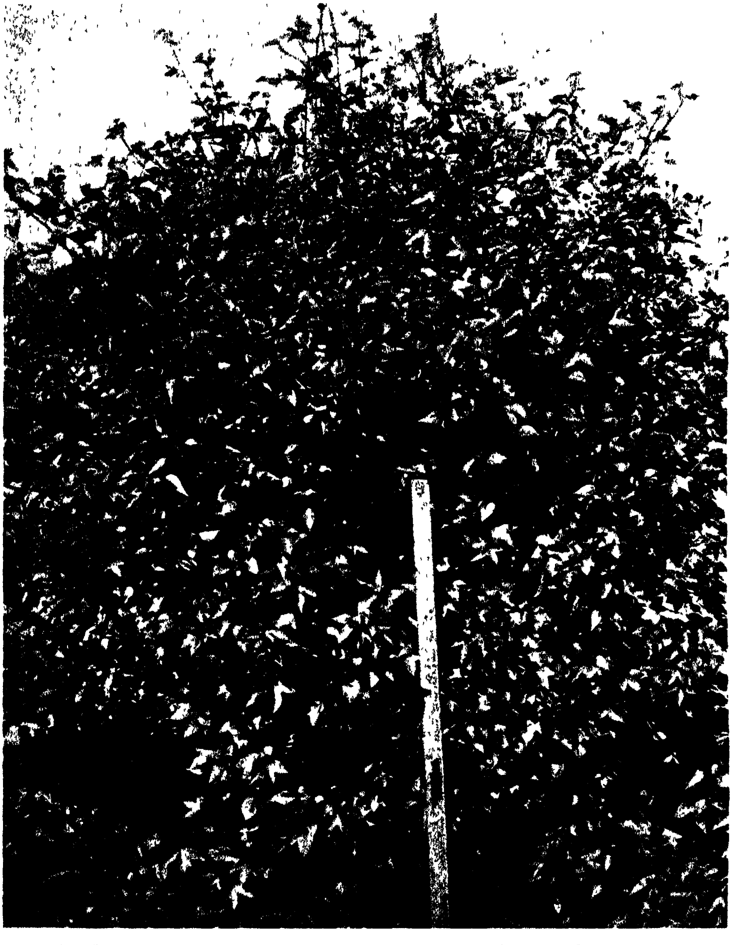


FIGURE 4.—First-generation plant of the interspecific hybrid *Gossypium armourianum* × *G. thurberi*, grown at Riverside, Calif. Note the intermediate condition in regard to compactness of branching and lobation of the leaves, as compared with the expression of these characters in the parent species (figs. 1 and 2).

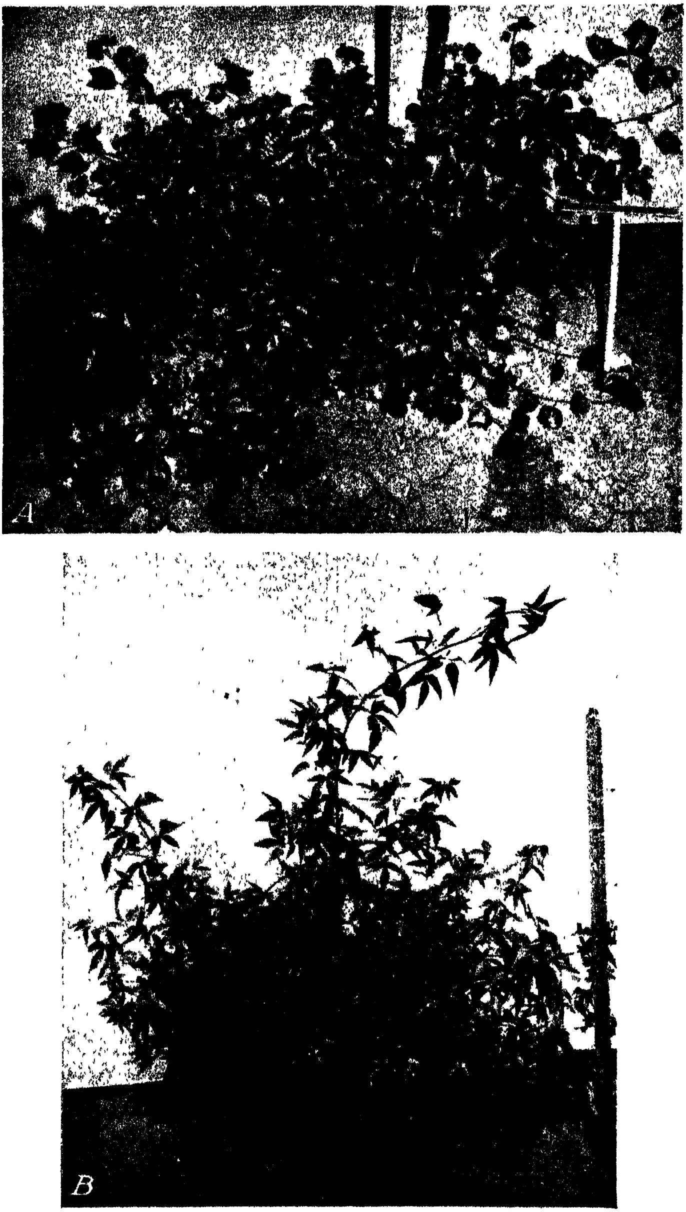


FIGURE 5.—Second-generation plants of *Gossypium armourianum* × *G. thurberi*: *A*, A plant that approaches the *G. armourianum* parent in its relatively compact habit and small, very shallowly lobed leaves; *B*, a plant that approaches the *G. thurberi* parent in its open habit and large, deeply lobed (pedate) leaves.

TABLE 1.—*Contrasting characters of Gossypium armourianum and G. thurberi and expression of these characters in F_1 and segregation in respect to them in F_2 of the hybrid G. armourianum × G. thurberi*

Character and expression	*G. armourianum*	*G. thurberi*	*G. armourianum × G. thurberi*			
			F_1	Classification of F_2		
				Both progenies as one array	Progeny W8	Progeny W9
Twigs, shape of cross section:						
Circular	×			15	8	7
Intermediate			×	30	17	13
Quadrangular		×		13	7	6
Leaf blade, thickness:						
Thick	×		×	24	12	12
Intermediate				30	17	13
Thin		×		5	3	2
Leaf blade, surface						
Shiny	×		×	20	11	9
Intermediate				28	16	12
Dull		×		11	5	6
Leaf blade, lobation						
None or very slight	×			14	10	4
Intermediate				17	9	8
Very deep		×	×	28	13	15
Leaf blade, apex:						
Obtuse or acutish	×			12	8	4
Short-acuminate			×	23	11	12
Attenuate-acuminate		×		24	13	11
Leaf blade, length:						
4 cm or less	×			19	11	8
5 to 9 cm			×	40	21	19
10 cm or more		×		0	0	0
Midlobe, basal constriction:						
None	×			32	21	11
Shallow			×	18	10	8
Deep		×		9	1	8
Peduncle, nectaries						
None	×			22	16	6
Present but small				21	9	12
Present, large		×	×	14	6	8
Bractlets, persistence.						
Caducous when bud is very small	×		×	39	17	22
Less caducous				14	11	3
Persistent until anthesis		×		1	0	1
Bractlets, length·						
5 mm or shorter	×			45	21	24
5 to 9 mm		×	×	9	7	2
10 mm or longer		×		0	0	0
Calyx margin.						
Conspicuously dentate	×		×	35	19	16
Slightly dentate		×		16	9	7
Entire or merely undulate		×		3	0	3
Petal color						
Pale yellow	×			23	6	17
Ochroleucous			×	16	11	5
White		×		13	9	4
Petal spot·						
Distinct	×	×		16	7	9
Very faint			×	13	5	8
None	×	×	×	23	14	9
Pollen color.						
Clear yellow	×			10	1	9
Pale yellow			×	12	1	11
Cream		×	×	29	24	5
Stigmas, color:						
White	×		×	31	14	17
Greenish white				15	10	5
Green		×		5	1	4
Stigmas, oil glands, color:						
Red brown	×		×	29	18	11
Yellowish				20	7	13
Green		×		2	0	2

In regard to certain characters in which F_1 resembled *Gossypium armourianum*, viz, thick leaf blades, shiny leaf surface, caducous bractlets, dentate calyx, white stigmas, and brown oil glands of the stigmas, F_2 plants that resembled *G. armourianum* were much more numerous than those that resembled *G. thurberi*. The marked dominance of deciduous bractlets (fig. 3, *A*) is noteworthy in view of the fact that in all cultivated cottons and in nearly all of the wild species they persist until the boll is mature. The bractlets in *G. thurberi* (fig. 3, *B*), although much smaller than in any cultivated cotton, are equally persistent.

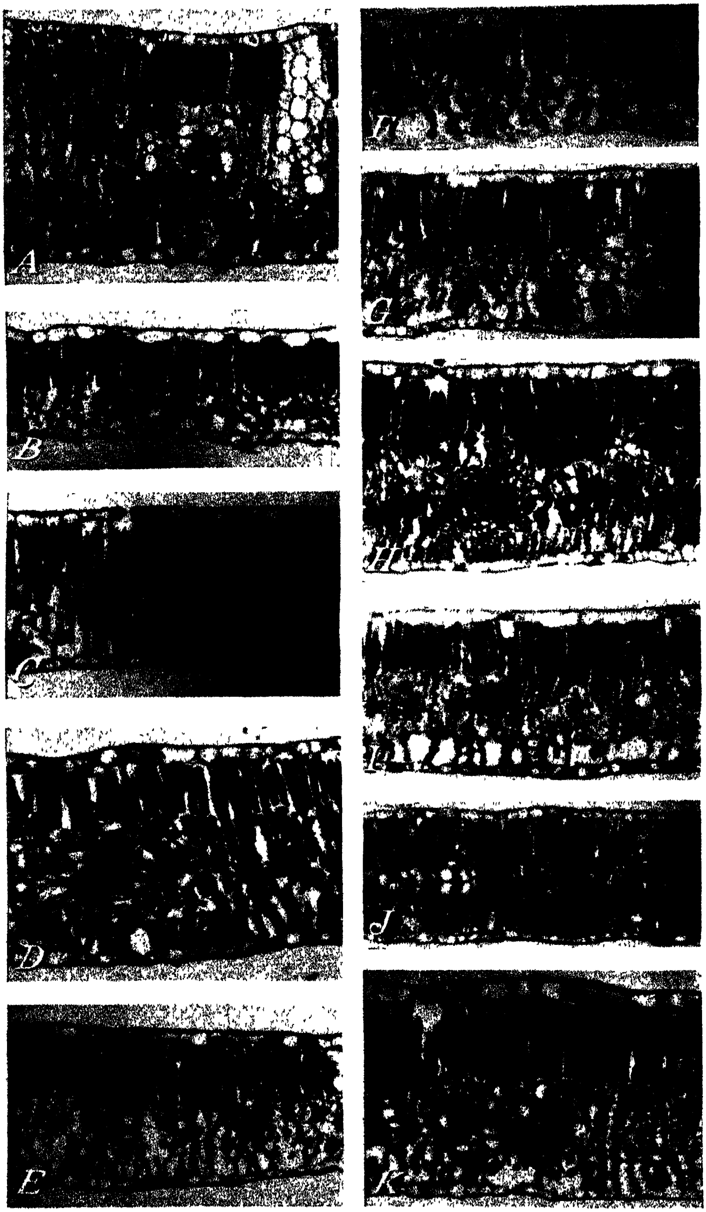
Characters in respect to which F_1 and a considerable majority of F_2 plants resembled *Gossypium thurberi* more closely than *G. armourianum* were pronounced lobation and acuminate apex of the leaf blades. No pronounced dominance of either parent is shown by the F_2 distribution for presence or absence of large extrafloral nectaries on the peduncle, although presence was dominant in F_1. The color of the petals was intermediate in F_1, but the F_2 population shows a tendency to dominance of the pale yellow of *G. armourianum*. In intraspecific crosses among cultivated cottons, yellow is almost completely dominant in F_1 and the segregations in F_2 indicate a ratio of 3 (full and paler) yellow to 1 white (*4*).

The evidence from the F_2 population, as well as from F_1, indicates that the number of dominant genes contributed by the *Gossypium armourianum* parent was larger than the number of dominant genes contributed by the *G. thurberi* parent.

It remains to consider the characters in respect to which the two F_2 progenies showed pronounced differences in their frequency distributions. These are leaf lobation, midlobe constriction, peduncle nectaries, petal color, petal spot, and pollen color. The F_1 parent individuals, W8 and W9, have not been observed to differ markedly in respect to the first four characters, so no explanation of the differences between the F_2 progenies is forthcoming at present. In view of the small size of the populations, the differences may not be significant.

As regards petal spot, there was segregation in F_1. Plant W8 had no spot on the petals of most of the flowers, whereas nearly all of the flowers on plant W9 had faint spots. The individual difference in petal spot in F_1 is accounted for by the fact that both *Gossypium armourianum* and *G. thurberi* are heterozygous in the wild, as well as in the populations grown at Riverside, for presence of a faint petal spot as contrasted with its absence. The *G. armourianum* parent of both W8 and W9 had spotless petals, but different individuals of *G. thurberi* served as parents of W8 and W9 and these were recorded as spotless and spotted, respectively. This probably explains the greater proportion of spotted flowers in F_2 progeny W9 (table 1). In view of the weak expression of this character in both parent species, classification by phenotypes is very uncertain and it is not unlikely that in reality all of the parents of the original crosses were heterozygous.

The F_1 plants W8 and W9 differed also in pollen color, the pollen of W8 being cream-colored and that of W9 being pale yellow. This segregation in F_1 is inexplicable, no appreciable variation in color of the pollen having been observed in the parent species. So far as has been observed, the pollen is always apricot yellow in *Gossypium armourianum* and uniformly cream-colored in *G. thurberi*. It will be noted



Cross sections of leaf blades of *Gossypium*, showing thickness and structure of the mesophyll, × 96: *A*, *G. armourianum*; *B*, *G. thurberi*; *C*, *G. armourianum* × *G. thurberi*, F$_1$, plant W9; *D*, the backcross (*G. armourianum* × *G. thurberi*, F$_1$) × *G. armourianum*, F$_1$; *E*, the backcross (*G. armourianum* × *G. thurberi*, F$_1$) × *G. thurberi*, F$_1$; *F*, W8, F$_2$, plant 2; *G*, W8, F$_2$, plant 28; *H*, W8, F$_2$, plant 12; *I*, W8, F$_2$, plant 14; *J*, W9, F$_2$, plant 9; *K*, W8, F$_2$, plant 25.

(table 1) that in the progeny of the F_1 plant that has cream-colored pollen (W8) a large majority of the F_2 individuals resemble the parent, whereas in the progeny of the F_1 plant with pale-yellow pollen (W9) the number of plants with yellow pollen exceeds the number with cream-colored pollen.

In intraspecific crosses among American cultivated cottons, presence of petal spot and yellow pollen are dominant to absence of spot and cream-colored pollen, respectively, with definite 3:1 segregation in F_2. Reference to table 1, columns 5 to 7, shows no indication that these small populations segregated in a definite ratio in respect to these characters; but, especially if the F_2 population as one array is considered (column 5), absence of petal spot and cream-colored pollen would appear to be imperfectly dominant. This is the reverse of what would be expected from the behavior of intraspecific crosses and of hybrids between 26-chromosome species, such as *Gossypium hirsutum* × *G. barbadense* (*2; 3; 5, pp. 22, 26; 6*).

LEAF ANATOMY OF THE PARENT SPECIES AND THEIR HYBRID

CHARACTERS INVESTIGATED, MATERIAL, AND METHODS

Little has been published hitherto on the gross morphology of hybrids involving American wild cottons, and there has been no report concerning the histology of such hybrids. There is much variation in the shape and size of the leaves on individual plants of *Gossypium thurberi*, but a previous histological survey of a number of species of *Gossypium* (*9*) showed that certain anatomical features of the leaf blade and petiole are fairly constant in this species. Epidermal characters are among such relatively constant features in *G. thurberi*, although differences in the pubescence and stomata of the leaves of this species and those of *G. armourianum* are quantitative rather than qualitative. On the other hand, *G. armourianum* and *G. thurberi* are characterized by striking differences in the structure of their mesophyll and petioles. This fact, coupled with the comparative difficulty of making and photographing preparations showing the epidermal surface, suggested that a comparison of the anatomy of hybrids between *G. armourianum* and *G. thurberi* might well begin with an examination of sections of their leaf blades and petioles.

All of the plants upon which this report is based were grown in a field at the Rubidoux Laboratory, Riverside, Calif. From each plant, fully matured leaves of typical vegetative shoots grown in exposed positions were chosen for study. Segments for sectioning were cut from approximately the center of the lamina and the middle of the petiole of each of the leaves. This material was killed in chromacetic formalin, dehydrated with *n*-butyl alcohol, embedded in paraffin, sectioned with a rotary microtome, stained with safranine, and mounted in balsam.

STRUCTURE OF THE MESOPHYLL

The Parent Species

The leaves of *Gossypium armourianum* (pl. 1, *A*) commonly have all of the chlorenchyma in the form of palisade tissue. Occasionally some of the chlorenchyma cells in the interior of the leaves are not greatly elongate and tend to be irregular in form, but typical well-developed palisade tissue always adjoins both the upper and the lower epidermis. Since as many as six layers of palisade cells are frequently present in the leaves of *G. armourianum*, the mesophyll of this species is characteristically thick as well as dense.

In contrast to the leaves of *G. armourianum*, those of *G. thurberi* (pl. 1, *B*) are relatively thin and distinctly bifacial. Their palisade tissue is restricted to the layer of cells subjacent to the upper epidermis of the leaf. The remainder of the chlorenchyma is composed of several cell layers of rather loose spongy parenchyma.

FIRST-GENERATION HYBRIDS AND BACKCROSSES

The two plants of *Gossypium armourianum* × *G. thurberi* F_1 designated as W8 and W9 have the leaves intermediate between those of the parental species in respect to thickness and mesophyll structure (pl. 1, *C*). Their mesophyll is characterized by a well-developed layer of palisade parenchyma subjacent to the upper epidermis; a layer in part spongy parenchyma but predominantly weak palisade parenchyma, which adjoins the lower epidermis; and several intervening layers of more or less elongate cells.

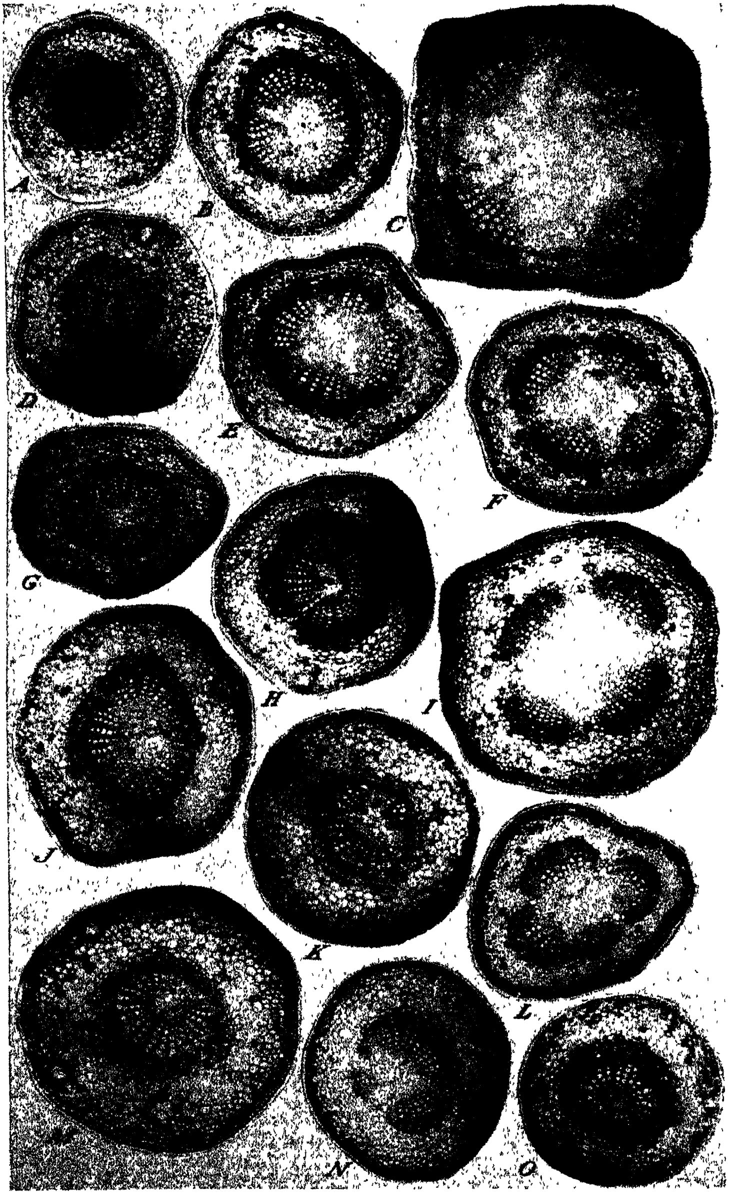
Mesophyll structure in a plant of the backcross (*Gossypium armourianum* × *G. thurberi* F_1) × *G. armourianum* (pl. 1, *D*) is intermediate between that of the F_1 hybrid and that of *G. armourianum*. The mesophyll approaches that of *G. armourianum* in being more compact and having a greater number of cell layers than that of *G. armourianum* × *G. thurberi* F_1, but it is neither quite so compact nor quite so thick as that of *G. armourianum*.

The structure of the mesophyll of the reciprocal backcross (*Gossypium armourianum* × *G. thurberi* F_1) × *G. thurberi* (pl. 1, *E*) is intermediate between that of *G. armourianum* × *G. thurberi* F_1 and that of *G. thurberi*. In this backcross plant the mesophyll is slightly thicker than that of *G. thurberi* because of a somewhat greater development of spongy parenchyma. The spongy parenchyma of the backcross plant contains many cells that are considerably more elongate than in the corresponding tissue of *G. thurberi*: but the spongy parenchyma is little if any more compact in the backcross than in *G. thurberi*, since it contains numerous large intercellular spaces.

SECOND-GENERATION HYBRIDS

An examination of leaves representing 29 plants from selfed seed of W8 and 25 plants from selfed seed of W9 showed that the mesophyll of F_2 plants (pl. 1, *F, G, H, I, J, K*) varies considerably. The F_2 population comprised plants in which the mesophyll closely resembled, respectively, that of *Gossypium thurberi* (pl. 1, *F*, as compared with pl. 1, *B*), *G. armourianum* × *G. thurberi* F_1 (pl. 1, *G*, as compared with pl. 1, *C*), the backcross (*G. armourianum* × *G. thurberi* F_1) × *G. armourianum* (pl. 1, *H*, as compared with pl. 1, *D*), and the backcross (*G. armourianum* × *G. thurberi* F_1) × *G. thurberi* (pl. 1, *I*, as compared with pl. 1, *E*). In other F_2 plants the mesophyll structure differed from all of the foregoing. Noteworthy among such additional types of mesophyll structure are those shown in plate 1, *J* and *K*. Plate 1, *J*, represents a cross section of a relatively thin leaf in which the chlorenchyma is composed of four layers of palisade cells; plate 1, *K*, represents a relatively thick leaf in which spongy parenchyma is present.

If the F_2 population is considered as a whole, it may be observed that the mesophyll contains a considerably larger proportion of palisade



Cross sections of petioles of *Gossypium*, taken at approximately the midpoint, × 33: *A*, *G. armourianum*; *B*, *G. armourianum* × *G. thurberi*, F_1, plant W9; *C*, *G. thurberi*; *D*, the backcross (*G. armourianum* × *G. thurberi*, F_1) × *G. armourianum*, F_1; *E*, the backcross (*G. armourianum* × *G. thurberi*, F_1) × *G. thurberi*, F_1; *F*, W9, F_2, plant 17; *G*, W9, F_2, plant 4; *H*, W8, F_2, plant 6; *I*, W8, F_2, plant 21; *J*, W9, F_2, plant 25; *K*, W9, F_2, plant 12; *L*, W9, F_2, plant 18; *M*, W9, F_2, plant 11; *N*, W8, F_2, plant 19; *O*, W9, F_2, plant 20.

tissue than occurs in the mesophyll of the leaves of *Gossypium thurberi.* Typical loose spongy parenchyma, similar to that adjoining the lower epidermis in the leaves of *G. thurberi,* is present in the mesophyll of but 13 of the 54 F_2 plants examined. In the remaining 41 F_2 plants the mesophyll is in all cases more compact than that of *G. thurberi,* and in many instances denser than that of *G. armourianum* × *G. thurberi* F_1. Typical palisade parenchyma adjoins both the upper and lower epidermis of the leaves of 23 of these plants.

STRUCTURE OF THE PETIOLE

The Parent Species

Cross sections taken at approximately the midpoint of the petiole of *Gossypium armourianum* are oval or round in outline and have a continuous ring of fibrovascular tissue (pl. 2, *A*). The diameter of petioles of *G. armourianum* is among the smallest diameters occurring in the genus *Gossypium.*

Analogous sections of the petioles of *G. thurberi* (pl. 2, *C*) are commonly quadrangular in outline and of considerably larger diameter than the petioles of *G. armourianum.* Fibrovascular tissue in cross sections taken at the midpoint of petioles of *G. thurberi* occurs chiefly in four conspicuous bundles underlying the corners of the petiole. Additional fibrovascular tissue is often developed to a slight extent between the four major bundles, but it never occurs in sufficient amount to produce a continuous fibrovascular cylinder of fairly uniform width like that in *G. armourianum.*

First-Generation Hybrids and Backcrosses

Cross sections of the petioles of *Gossypium armourianum* × *G. thurberi* F_1 (pl. 2, *B*), though intermediate in size between those of the parents, bear a somewhat closer structural resemblance to *G. armourianum* than to *G. thurberi.* Their outline tends to be more angular than that of the petioles of *G. armourianum* but is distinctly rounder than that of the petioles of *G. thurberi.* The fibrovascular tissue occurs in a ring, which, except for somewhat larger size and a slightly less uniform distribution of vessels, resembles the fibrovascular ring in analogous sections of petioles of *G. armourianum.*

The size of cross sections taken at approximately the midpoint of the petiole of the backcross (*Gossypium armourianum* × *G. thurberi,* F_1) × *G. armourianum* (pl. 2, *D*) is nearly the same as that of analogous sections of the petioles of *G. armourianum* × *G. thurberi* F_1, with an approach to the *G. armourianum* parent in their smaller fibrovascular ring and slightly more rounded outline. Likewise, sections taken at about the midpoint of the petioles of the reciprocal backcross (*G. armourianum* × *G. thurberi* F_1) × *G. thurberi* (pl. 2, *E*) are of a size approximating that of analogous sections of petioles of *G. armourianum* × *G. thurberi* F_1 rather than of *G. thurberi.* An approach to the latter species is shown, however, by a more angular outline than in F_1 and by the uneven distribution of vessels within the fibrovascular ring, suggestive of four contiguous bundles rather than a continuous cylinder.

Second-Generation Hybrids

Individual plants in the F_2 population of *Gossypium armourianum* × *G. thurberi* differ greatly in the size, angularity, and distribution of fibrovascular tissue in their petioles. Comparable cross sections of the petioles of these F_2 plants (pl. 2, *F–O*) range in size from about that of cross sections of petioles of *G. armourianum* (pl. 2, *G*, *O*, as compared with pl. 2, *A*) to nearly that of cross sections of petioles of *G. thurberi* (pl. 2, *I*, *M*, as compared with pl. 2, *C*), but mostly approximate in size the cross sections of *G. armourianum* × *G. thurberi* F_1 (pl. 2, *B*). The diameter of sections of petioles of the F_2 plants shows no correlation with their outline nor with the arrangement of fibrovascular tissue within the petiole. Sections of either relatively small diameter (pl. 2, *G*, *O*) or relatively large diameter (pl. 2, *I*, *M*) may be rounded (pl. 2, *M*, *O*) or somewhat angular (pl. 2, *G*, *I*) and may have their fibrovascular tissue disposed in a ring (pl. 2, *G*, *M*) or in several distinct bundles (pl. 2, *F*, *I*). Cross sections of the petioles of some of the F_2 plants rather closely resemble those of *G. armourianum* (pl. 2, *O*, as compared with pl. 2, *A*), *G. thurberi* (pl. 2, *I*, as compared with pl. 2, *C*) or *G. armourianum* × *G. thurberi* F_1 (pl. 2, *H*, as compared with pl. 2, *B*). Others show arrangements of fibrovascular tissue differing from those found in either parent species, or in F_1 (pl. 2, *K*, *L*, *N*).

The arrangement of vascular tissue in the petioles of the F_2 population as a whole shows greater resemblance to *Gossypium armourianum* or to *G. armourianum* × *G. thurberi* F_1 than to *G. thurberi*. A tendency to dominance of characters of *G. armourianum*, rather than of *G. thurberi*, appears therefore in the anatomy as well as in the gross morphology of the F_2 plants. Four comparatively large and widely spaced fibrovascular bundles, such as characterize the cross sections taken at approximately the midpoint of the petioles of *G. thurberi*, are present in analogous sections of petioles of only 12 of the 54 F_2 plants examined. Moreover, in the sections of petioles from these 12 plants the fibrovascular bundles are closer to one another than they are in comparable sections of the petioles of *G. thurberi*. The F_2 population, as a whole, also shows greater resemblance to *G. armourianum* or to *G. armourianum* × *G. thurberi* F_1 than to *G. thurberi* in that the petioles are much more frequently roundish or slightly angular than distinctly quadrangular.

SIGNIFICANCE OF THE ANATOMICAL FINDINGS

The number of plants in the F_2 population was too small to furnish a reliable basis for conclusions as to the inheritance ratios of characters in hybrids between wild American cottons, but is large enough to be indicative of the trends in structural features of the leaves. Such data are of interest, not only because of the bearing they may have on the origin of certain characteristics of cultivated American cottons, but also, in view of the relation between the morphology and the physiology of leaves, because of their possible significance to the plant breeder.

Relationships between morphological and physiological characteristics of the lamina have received much attention from ecologists. Consequently the influence of various environmental factors, particularly illumination, on structure and function of the epidermis and mesophyll of the leaf has been well established for many plants. Despite the recognized variability of leaves of a given species when grown under varying conditions, it is not uncommon for different species of the same genus to show structural differences that are sufficiently constant to be employed for taxonomic purposes. As may be seen from the literature cited by Halma (*1*), morphological differences in the mesophyll of different species of the same genus have been correlated with physiological differences between such species. It has been found that in some genera the growth rate and productiveness of the species show a close positive correlation with the relative development of palisade tissue in the mesophyll.

The existence of correlations between the physiological behavior of the several species of *Gossypium* and differences in the development of palisade tissue in their leaves has not been determined. Consequently, the relationships established for other genera can only be regarded as suggestive in the case of *Gossypium*. However, since in other genera the desirability, from the practical standpoint, of obtaining plants with a relatively large proportion of palisade tissue is apparent, the possible existence in *Gossypium* of a correlation between desired physiological characteristics and abundant palisade development should not be overlooked. In view of this possibility, it is interesting that 41 of the 54 F_2 plants of *G. armourianum* × *G. thurberi* have a larger proportion of palisade tissue in their mesophyll than occurs in *G. thurberi*, the parental species possessing typical spongy parenchyma. The fact that there is a much higher proportion of palisade tissue in the mesophyll of the leaves of *G. armourianum* Kearney and *G. harknessii* Brandeg. than in the leaves of *G. klotzschianum* Anderss., *G. davidsonii* Kellogg, *G. thurberi* Tod., and *Erioxylum aridum* Rose and Standl. (*G. aridum* Skovsted) suggests that of these American wild cottons the two first-named species may be the most useful for breeding purposes.

Regardless of whether or not a correlation between palisade development and desirable physiological characteristics is found to exist in *Gossypium*, the tendency of hybrids between *G. armourianum*, a species characterized by centric leaves, and *G. thurberi*, a species with bifacial leaves, to have a more compact mesophyll structure than that occurring in the parent with bifacial leaves is of significance from the viewpoint of phylogeny. Since the leaves of the American cultivated cottons commonly have a layer of spongy parenchyma adjoining their lower epidermis (*9*), it may be inferred that at least one of the species involved in the origin of the American cultivated cottons possessed a mesophyll with typical spongy parenchyma. Among the American wild cottons a mesophyll of this type is known to occur in *G. davidsonii*, *G. klotzschianum*, and *Erioxylum aridum* as well as in *G. thurberi*. On the other hand, the mesophyll of *G. harknessii* resembles that of *G. armourianum*. Nothing is known concerning the mesophyll structure of the Mexican wild species *G. trilobum* (DC) Kearney (*Ingenhouzia triloba* DC) and *G. gossypioides* (Ulbr.) Standl. (*Selera gossypioides* Ulbr.) or of the Peruvian wild species *G. raimondii* Ulbr.

The structure of the petiole has often been profitably employed for taxonomic purposes, especially as an aid in specific diagnosis, but efforts to correlate petiole structure and the physiological characteristics of leaves are apparently wanting. Hence a comparison of analogous sections of petioles of hybrids between *Gossypium armourianum* and *G. thurberi* is of value at present chiefly from the taxonomic standpoint. As indicated above, petioles that are roundish or only slightly angular predominate in hybrids between *G. armourianum*, a species with petioles that are roundish in cross section, and *G. thurberi*, a species with petioles that are distinctly quadrangular in cross section. There is also a marked tendency for the fibrovascular tissue in the petioles of these hybrids to occur more frequently in a closed cylinder, as in *G. armourianum*, than in separate fibrovascular bundles, as in *G. thurberi*.

At present too little is known concerning the structure of petioles of the cultivated American cottons to afford a basis of speculation as to what the foregoing indications of dominant and recessive characters may suggest as to the type or types of petioles possessed by the species involved in the origin of the cultivated American cottons. However, in view of the reported similar cytological behavior of such morphologically distinct species as *G. armourianum* and *G. thurberi* (*10*), it would seem that in seeking to establish the phylogenetic origin of the cultivated American cottons their morphological characteristics should be considered in conjunction with the dominance relations of morphological characters in hybrids involving species with 13 chromosomes.

SUMMARY

Two wild, lintless, 13-chromosome species of cotton, *Gossypium armourianum* and *G. thurberi*, both native in North America, were crossed in an experiment at Riverside, Calif.

The resulting hybrid proved to be much more fertile than any other hybrid that has been obtained by crossing two truly wild species of *Gossypium*. F_2 populations, totaling 64 individuals, were grown in 1937 and 1938.

Interspecific hybrids in *Gossypium* are likely to prove important in contributing to a better understanding of the phylogeny and taxonomy of the genus; and study of their morphology and cytological behavior may help to solve the problem of the origin of the cultivated American cottons with 26 chromosomes. There is also a possibility that they may be utilized eventually in breeding cottons superior in resistance to drought, diseases, and insect pests.

The first part of this paper deals with the gross morphological characters of the parent species and their inheritance in the hybrid.

Some of the more conspicuous contrasting characters of *Gossypium armourianum* and *G. thurberi*, and their expression in the first generation of the hybrid (F_1), are stated. The hybrid resembles the *G. armourianum* parent in six of these characters and the *G. thurberi* parent in two characters. In six characters, the hybrid is more or less intermediate between the parent species.

The classifications of the second-generation (F_2) population in respect to these characters are also presented. There is strong indication of simple Mendelian or monohybrid segregation in only one character, shape of cross section of the twigs. There is close approach to a 1:2:1 ratio in this instance.

The tendency to dominance of a greater number of genes from *Gossypium armourianum* than from *G. thurberi* is evident in F_2, as well as in F_1.

A reversal of the dominance tendencies of the characters petal spot and pollen color, as shown in intraspecific crosses and in hybrids between different species of cultivated American cottons, is indicated in *Gossypium armourianum* × *G. thurberi* F_2. The population is much too small, however, to warrant a definite conclusion on this point.

No detailed comparison of the anatomical characters of an interspecific hybrid in *Gossypium* with that of its parents appears to have been published previously. The anatomical features of the mesophyll and the petiole alone are considered in this paper.

As in the gross morphological features, the hybrid resembles *Gossypium armourianum* more closely than *G. thurberi* in its histological characters.

The compact mesophyll and closed fibrovascular cylinder of the petiole of *Gossypium armourianum* are partly dominant to the relatively loose mesophyll and separate fibrovascular bundles of *G. thurberi*.

Consideration is given to the probable physiological advantage of a compact mesophyll with highly developed palisade tissue, such as characterizes *Gossypium armourianum*, and to the possible significance, in plant breeding, of leaf structure of this type.

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SOFT ROT OF PUMPKIN AND WATERMELON FRUITS CAUSED BY PYTHIUM ULTIMUM[1]

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INTRODUCTION

In connection with studies of a bacterial soft rot of Zucchini pumpkin fruits (*Cucurbita pepo* L. var. *condensa* Bailey), briefly described in a preliminary note by Ark and Tompkins (*2*)[3], a crate of field-infected fruits was shipped from Encinitas in southern California to Berkeley, Calif., in January 1936. Although some of these fruits upon examination showed bacterial soft rot, most of them were obviously involved in a fungus type of decay as revealed externally by soft, water-soaked areas partly covered with white mycelium. The causal fungus was identified as *Pythium ultimum* Trow and its pathogenicity proved in inoculation tests. Later the disease was found on Mammoth Summer Crookneck pumpkin fruits at Salinas and on Klondyke watermelon fruits (*Citrullus vulgaris* Schrad.) obtained in a Berkeley market. This paper deals with symptoms of the disease, the causal organism, and host range.

REVIEW OF LITERATURE

Species of *Pythium* have frequently been recorded as pathogens of cucurbit fruits.

McRae (*28*) reported from India that strains of *Pythium* were associated with decay of *Luffa acutangula* Roxb., *L. aegyptiaca* Mill., *Trichosanthes anguina* L., and *Lagenaria vulgaris* Ser. Parisi (*31*) noted the occurrence of *P. debaryanum* on fruits of chayote (*Sechium edule* Swartz). Blossom-end decay of watermelons, caused by a fungus resembling *Pythium artotrogus* DeBary, was described by Drechsler (*6*) in 1923. He also found a fruit decay in the field associated with *P. debaryanum*, and described a disease of cucumber (*Cucumis sativus* L.) to which he gave the name cottony leak (*7*).

In 1928, Mitra and Subramaniam (*30*) also reported a fruit rot of various cultivated cucurbits in India, caused by *Pythium aphanidermatum* (Eds.) Fitz. Infection was observed on cucumber, *Cucumis melo* L. var. *momordica*, white-flowered gourd (*Lagenaria leucantha* Rusby), *Luffa acutangula, L. aegyptiaca*, balsam-pear (*Momordica charantia* L.), serpent or snakegourd (*Trichosanthes anguina*), and *T. dioica*. The symptoms were in close agreement with those described by Drechsler for cucumber (*7*). The disease occurred in

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[3] Italic numbers in parentheses refer to Literature Cited, p. 473.

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the field during and after rains. Fruits on or near the soil level were readily attacked, and the disease was also observed to spread in storage.

Pythium myriotylum Drechsler, the cause of a decay of cucumber and watermelon fruits in the Southeastern States, was considered by Drechsler (*9*) to be one of the most destructive members of the genus. It is favored by high humidity and usually occurs in southern latitudes. Other species capable of rotting watermelons included *P. periplocum* Drechsler and *P. acanthicum* Drechsler. *P. debaryanum* was considered by Walker and Weber (*44*) to be one of the causes of blossom end rot of watermelon fruits in Florida. The disease is more commonly found on melons about one-fourth grown. Kheswalla (*24*) called attention to a rot of watermelons in Baluchistan caused by *P. aphanidermatum.*

TABLE 1.—*Plants reported susceptible to Pythium ultimum by various investigators*

Host	Locality	Investigator
Apium graveolens L.	United States	Alexander, Young, and Kiger (*1*).
Arachis hypogea L.	Union of South Africa	Wager (*40, 41*)
Beta vulgaris L.	United States	Poole (*34, pp. 807–814*).
B. vulgaris var. *crassa* Alef	Canada	Jones (*23*).
B. vulgaris var. *macrorhiza* Stev.	do	Do
	United States	Alexander, Young, and Kiger (*1*).
Brassica oleracea L.	England	Trow (*39*).
	United States	Drechsler (*8*). Harter and Whitney (*15*). Walker (*43*).
	Union of South America	Wager (*41*).
B. rapa L.	United States	Poole (*34*).
Callistephus chinensis Nees	Union of South Africa	Wager (*40, 41, 42*)
Carica papaya L.	do	Wager (*40, 41*).
Cupressus sp.	Rhodesia	Hopkins (*17*).
Dahlia sp.	Union of South Africa	Wager (*41*)
Daucus carota L.	Canada	Jones (*23*).
Dioscorea batatas Decne.	France	Labrousse (*25*).
Gossypium sp.	United States	Arndt (*3*).
Ipomoea batatas (L.) Lam.	Union of South Africa	Wager (*40, 41*).
	United States	Drechsler (*10*) Harter and Whitney (*15, 16*). Poole (*33, 34*).
Lactuca sativa L.	do	Alexander, Young, and Kiger (*1*).
Lepidium sativum L.	England	Trow (*39*).
Lycopersicum esculentum Mill.	United States	Alexander, Young, and Kiger (*1*). Horsfall (*18*). Wilson and Tilford (*45*).
	New Zealand	Brien and Chamberlain (*4*).
Medicago sativa L.	United States	Buchholtz and Meredith (*5*).
Nasturtium sp.	Union of South Africa	Wager (*41*).
Nicotiana tabacum L.	United States	Alexander, Young, and Kiger (*1*).
Papaver nudicaule L.	Union of South Africa	Wager (*40, 41*).
Phaseolus vulgaris L.	do	Wager (*41*).
	United States	Harter and Whitney (*15, 16*). Poole (*34*).
Picea engelmannii Engelm.	do	Rathbun-Gravatt (*35*).
Pinus banksiana Lamb.	do	Do
P. resinosa Ait.	do	Do.
Pisum sativum L.	Union of South Africa	Wager (*41*).
Raphanus sativus L.	United States	Poole (*34*).
Rheum rhaponticum L.	Union of South Africa	Wager (*40, 41*)
Solanum tuberosum L.	England	Trow (*39*).
	United States	Poole (*34*).
	Canada	Conners.[1] Jones (*23*).
Spinacea oleracea L.	France	Dufrénoy (*11*). Foex (*13*). Foex, Dufrénoy, and Labrousse (*14*). Labrousse (*25, 26, 27*).
	United States	Pirone et al. (*32*).
	Germany	Fabel (*12*).
Striga lutea	Union of South Africa	Wager (*40*).

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[1] CONNERS, I. L. THIRTEENTH ANNUAL REPORT OF THE CANADIAN PLANT DISEASE SURVEY 1933. Canada Dept. Agr., Expt. Farms Branch 128 pp. 1934. [Mimeographed.]

Hosts reported susceptible to *Pythium ultimum* are presented in table 1. Numerous other plants are reported as susceptible when grown in naturally infested soil inhabited by other soil fungi as well as by *Pythium ultimum*; for this reason these hosts are not listed. These hosts may be found by referring to the recent contributions of Horsfall (*18, 19, 20, 21*), Horsfall, Newhall, and Guterman (*22*) and Pirone, Newhall, Stuart, Horsfall, and Harrison (*32*) on the efficacy of various methods for the control of damping-off.

SYMPTOMS OF THE DISEASE

If in contact with or near the surface of wet soil, either during the rainy season or immediately after irrigation, Zucchini and Mammoth Summer Crookneck pumpkin fruits, irrespective of age or size, are susceptible to infection. The disease is favored by cool weather. Infection may occur on any part of the fruit but is found most commonly at either the stem or blossom end. The first symptom observed on naturally or artificially infected fruits consists of one or several slightly sunken, soft, water-soaked lesions of irregular size and shape. Under favorable conditions, there is a rapid enlargement of the lesions, accompanied by a marked sinking and softening of the underlying tissues. In advanced stages, the lesions are frequently covered with a dense, closely adpressed, white mycelium which is in marked contrast to the luxuriant, cottony growth of mycelium produced on cucumber fruits by *Pythium aphanidermatum* (*7*). The inherent firmness characteristic of healthy fruits is destroyed soon after infection; invaded fruits break open when handled, exposing the odorless, water-soaked, decaying tissues. Fruits may be completely rotted within 6 to 10 days after the appearance of the first symptoms of infection.

The conditions which predispose watermelons to infection under field or market conditions were not investigated. The symptoms on this host closely correspond with those on pumpkin fruits except that the lesions are army brown (*36*)[4] in color. Large quantities of free liquid are released when badly decayed fruits are broken in handling.

THE CAUSAL FUNGUS, PYTHIUM ULTIMUM

The fungus was readily obtained in pure culture by the usual tissue-planting technique (*29*) from 30 diseased pumpkins and 6 watermelon fruits.

Freehand cross and longitudinal sections of artificially infected tissues were stained with fast green and Magdala red. The fungus was both intercellular and intracellular.

MORPHOLOGY

Cultures grown on corn meal, oatmeal, and potato-dextrose agar developed a copious amount of white cottony aerial mycelium, filling the space between the slope and the wall of the tube after 4 days at room temperature. Cultures on steamed corn meal produced a profuse aerial mycelium, filling the basal portion of the Erlenmeyer flasks after 7 days. Hyphae were cylindrical, smooth, and nonseptate when young, measuring 1.7 μ to 6.2 μ in diameter, average 3.4 μ, and becoming septate with age.

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[4] Color determinations were made with the aid of Ridgway's manual (*36*)

Sporangia were present on the aerial growth in all cultures, being most numerous on potato-dextrose agar, moderately abundant on corn meal and oatmeal, and rare on steamed corn meal. Intramatrical sporangia were often produced, though never abundantly. Sporangia were spherical when acrogenous, measuring 14.5μ to 28.3μ in diameter, average 20.4μ (fig. 1, *D*). Occasional intercalary bodies were found which varied in size from 15.7μ in width and 19.3μ in length to 23.3μ $\times$27.3μ, average 19.6$\mu\times$23.9μ.

Tufts of mycelium from potato-dextrose agar were placed in pea broth and allowed to grow at room temperature. Transfers were made 4 days later to sterile distilled water and Petri's solution. Sporangia were produced abundantly in both solutions. All attempts to induce zoospore formation were futile, germination always occurring by the production of one to 5 germ tubes.

To study the formation of oogonia, antheridia, and oospores, the fungus was grown in clear media. Agar squares containing the organism were cut from 2-day-old potato-dextrose agar plates and transferred to Petri dishes containing corn-meal decoction agar and plain water agar. Oogonia, antheridia, and oospores were produced freely in these media in 3 to 4 days. Similar bodies were formed when tufts of mycelium were transferred from pea broth to sterile distilled water and Petri's solution.

Of the isolates from diseased cucurbit fruits, only those from Klondyke watermelon and Mammoth Summer Crookneck pumpkin produced oogonia. The isolate from Zucchini pumpkin used in the inoculation studies has never produced oogonia, even though a variety of media and procedures has been tried. Oogonia were largely intramatrical, acrogenous, spherical with a smooth thin wall, and measured 17.3μ to 24.2μ in diameter, average 20.2μ (fig. 2, *A*). Antheridia were generally androgynous, usually one to an oogonium, though occasionally two were present, in which case one was always of diclinous origin. Typical antheridia arose immediately below the oogonium on the oogonial stalk (fig. 2, *B*, *C*, *D*). They were somewhat clavate, short, upcurved, the tip in narrow contact with the oogonial wall. At maturity the contents of the oogonium differentiated into a peripheral layer, the periplasm, the remaining portion becoming the oosphere. A penetration tube was produced by the antheridium, permitting its contents to pass into the oosphere (fig. 2, *C*, *D*). After fertilization the oosphere further differentiated to form the oospore.

When mature the oospore was spherical, smooth, and thick-walled, not filling the oogonial cavity (fig. 2, *E*, *F*). The contents were somewhat granular. A reserve globule was present; frequently associated with it was a subspherical refringent body. The oospores measured 15.7μ to 22.9μ in diameter, average 17.4μ, including the oospore wall, which was approximately 1.4μ thick.

In addition to the reproductive bodies mentioned that formed in clear media, numerous swollen hyphal elements forming complex aggregations were found in contact with the bottom of the Petri dish (fig. 1, *A*, *B*). The hyphae were two to five times larger in diameter, and had denser contents than the parent hypha. They closely resembled the filamentous sporangia frequently found in *Pythium*, or the falcate bodies observed in *Pythium splendens*. Bits of agar containing these complexes were placed in sterile distilled water and

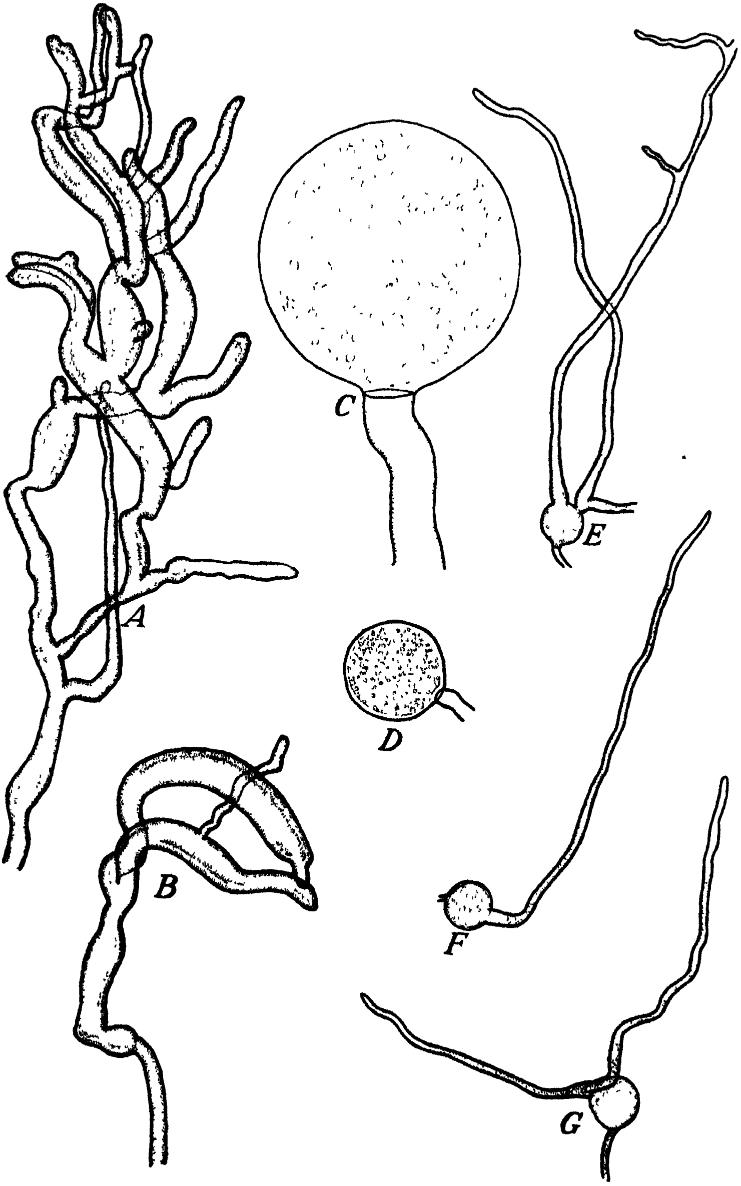

Figure 1.—*A, B,* Frustrated appressoria of *Pythium ultimum* (×675); *C,* reproductive body resembling oogonium (×1,250); *D,* terminal sporangium (×500); *E, F, G,* germination of oogonia by germ tubes (×250).

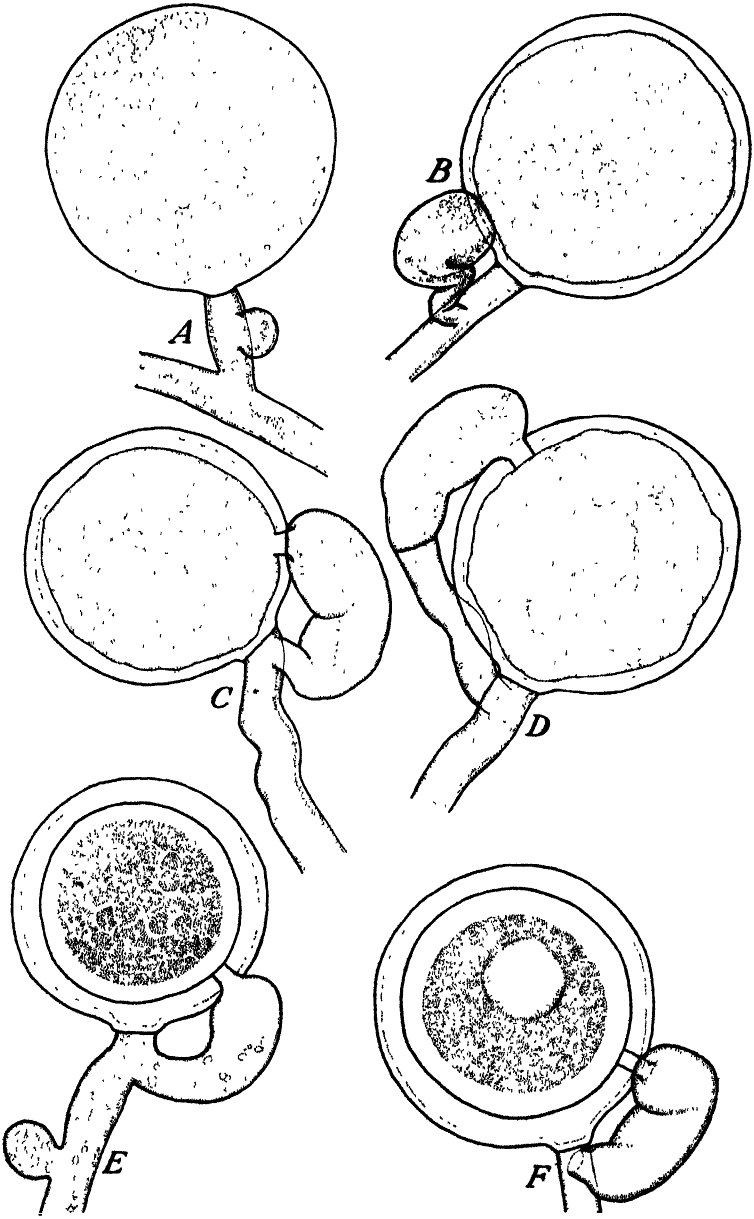

FIGURE 2.—Oospore formation in *Pythium ultimum*: *A*, Oogonium with antheridial element; *B*, antheridium in contact with oogonial wall; *C*, *D*, union of antheridial and oogonial contents; *E*, inmature oospore; *F*, mature oospore. *A–F* × 1,250.

Petri's solution in an attempt to stimulate zoospore development. Germination occurred by the production of normal hyphae arising from the tips of the enlarged threads; zoospores were never formed. Although these aggregations may be regarded as plasmatoögoses, the fact that they were produced only when the mycelium was in contact with the surface of the vessel, and never at random in the medium, leads the writers to consider them frustrated appressoria.

The causal organism corresponds quite closely with the fungus from *Lepidium sativum* described by Trow (*39*) as *Pythium ultimum*, and has been identified as that species by the writers. *P. ultimum* was originally described as a saprophyte; however, numerous reports in the literature refer to the species as an aggressive parasite of numerous hosts.

Of the species reported from cucurbits only *Pythium debaryanum* and *P. ultimum* produce spherical sporangia. Both have acrogenous spherical sporangia; the intercalary sporangia produced sparingly in *P. ultimum* are moderately abundant in *P. debaryanum*. Germination of the sporangia by germ tubes is common to both; however, zoospores are frequently produced by *P. debaryanum* but not by *P. ultimum*. The oogonia of *P. debaryanum* are smooth-walled and somewhat larger in diameter than those of *P. ultimum*. The intercalary oogonia frequently found in *P. debaryanum* are absent or extremely rare in *P. ultimum*. Several antheridia are produced in *P. debaryanum* which may be either diclinous or androgynous, but if the latter, they do not originate immediately below the oogonium as in *P. ultimum*. Mature oospores of both species lie free in the oogonial cavity. As indicated by Drechsler (*8*), a reserve globule is present in oospores of each species, though somewhat smaller in size in *P. ultimum*. A small refringent body is frequently associated with the globule; this is usually ellipsoidal in *P. debaryanum* and subspherical in *P. ultimum*. The oospore wall is approximately 1.0μ thick in *P. debaryanum* and 1.5μ in *P. ultimum*. Whether these two species should be maintained as separate entities is a moot question and should be further investigated.

A comparison of *Pythium ultimum* from fruits of *Citrullus vulgaris*, *Cucurbita pepo* var. *condensa*, roots of *Medicago sativa*, *Nicotiana tabacum* and *Spinacia oleracea* with Trow's original description and that reported by Wager (*40*) is presented in table 2.

It is apparent that the writers' isolates listed are identical and agree closely with the description of *Pythium ultimum* as set forth by Trow. Although two of the isolates from *Cucurbita pepo* failed to develop sexual organs it seems probable that they too should be referred to this species.

Reproductive bodies resembling oogonia were produced by the two cucurbit isolates which did not develop oospores (fig. 1, *C*). These were acrogenous, spherical, and thin-walled, and appeared intramatrically in clear media after 3 or 4 days. When a bit of agar containing the fungus was transferred to sterile distilled water the bodies germinated within an hour by the production of one to three germ tubes (fig. 1, *E, F, G*). These spherical bodies are considered oogonia rather than sporangia because similar structures were observed in cultures of isolates producing oogonia and oospores. The following procedure was used in this study.

TABLE 2.—*Spore measurements of Pythium ultimum isolations from Citrullus, Cucurbita, Medicago, Nicotiana, and Spinacia compared with measurements published by Trow and Wager*

Investigator and host	Oogonia			Oospores			Sporangia		
	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average
Trow (*39*)	μ	μ	μ	μ	μ	μ	μ	μ	μ
Lepidium sativum	19.6	22.9	20.6	14.7	18.3	16.3	12.0	28.0	20.0
Wager (*40*)									
Arachis hypogea	15.8	27.0	22.5	13.5	20.3	18.3	18.0	27.0	23.0
Carica papaya	14.4	27.0	21.3	11.3	20.7	17.0	22.5	22.5	22.5
Ipomoea batatas	18.0	27.0	22.8	13.5	21.6	18.0	15.8	27.5	23.8
Papaver nudicaule	16.7	27.0	22.4	12.2	21.2	18.1	13.5	29.7	23.9
Rheum rhaponticum	15.8	25.7	22.1	13.5	22.1	17.8	15.8	31.5	24.2
Striga lutea	13.5	22.5	18.9	11.3	20.7	16.0	13.5	22.5	20.6
Baarn (host unknown)	15.6	25.4	21.3	13.7	22.5	17.5	15.3	26.8	21.2
Writers (Tompkins, et al.)									
Citrullus vulgaris	17.3	23.6	20.3	15.7	22.0	17.2	17.3	26.8	20.3
Cucurbita pepo	17.6	24.2	20.2	15.8	22.9	17.7	15.1	28.3	20.9
Cucurbita pepo	---	---	---	---	---	---	15.9	27.6	20.4
Cucurbita pepo	---	---	---	---	---	---	14.5	26.6	20.0
Medicago sativa	14.4	23.2	20.9	11.6	19.7	17.0	16.4	28.5	21.1
Nicotiana tabacum	16.8	24.1	20.6	14.1	20.8	17.1	15.8	26.9	20.7
Spinacia oleracea	14.8	24.2	21.1	11.7	21.9	17.6	16.2	27.3	21.3

Strips of agar with the mycelium of an oospore-producing isolate were removed from a 3-day-old plate culture. The strips were cut to include a well-developed parent hypha bearing terminal oogonia on short lateral branches. Strips were chosen in which oospores were present at the proximal end of the hypha, nearest the origin of growth, while at the young distal end there were only unfertilized oogonia with no differentiation of oospores. The agar blocks were placed in sterile distilled water and within a short time the spherical bodies in the distal region germinated, producing one to three germ tubes. Germination of oospores was not observed. In comparable agar sections selected but not removed from the culture dish, oospores developed in the distal bodies within 24 hours. There can be little doubt that the reproductive structures on the younger portion of the hypha were oogonia. In appearance and behavior they appeared identical with the terminal bodies on the short lateral branches that developed in the nonoospore-producing isolates.

It seems probable that the failure of certain isolates to develop oospores is the result of failure to form antheridia. Since there is no evidence that a heterothallic condition occurs in the species it is suggested that the monosexuality exhibited by these strains may be genetic in nature.

TEMPERATURE RELATIONS

The relation of temperature to growth of the mycelium was determined for isolates from fruits of Zucchini and Mammoth Summer Crookneck pumpkin, and from Klondyke watermelon. In addition to the organisms from cucurbit fruits, isolates from tobacco, alfalfa, and spinach roots and a culture from the Centraalbureau voor Schimmelcultures, Baarn, Netherlands, received as *Pythium ultimum*, were included. The culture tubes (2.1 by 20 cm) used and the procedure followed in this study were those previously described by Tompkins et al. (*37*). To each tube, provided with a glass dam at the open end, 15 cc of Difco corn-meal agar (pH 6.0) was added and allowed to cool at a uniform depth with the tube in a horizontal position, the dam preventing the escape of the melted agar.

These tubes were inoculated near the dam at the open end with small squares of potato-dextrose agar containing the mycelium of the fungus cut from 2-day-old Petri-dish cultures. Inoculated tubes were left at room temperature for 24 hours, the extent of growth at the close of that period being indicated by a wax-pencil mark on the tube: subsequent measurements were made from this point. Two tubes of each culture were placed in a horizontal position in controlled temperature chambers at intervals of 3°, from 4° to 40° C.

Growth occurred at all temperatures, all isolates behaving similarly. Perceptible growth occurred at 4° and 40° C. only after 72 hours exposure. Average growth over 24-hour periods for the various temperatures is presented in table 3.

The minimum temperature is approximately 4°, the optimum 25° to 28°, and the maximum 40° C.

TABLE 3.—*Mycelial growth of Pythium ultimum on Difco corn-meal agar at various temperatures*

Isolate No	Average growth over 24-hour period at—												
	4° C	7° C	10° C	13° C	16° C	19° C	22° C	25° C.	28° C	31° C.	34° C.	37° C	40° C.
	Mm	*Mm*	*Mm*	*Mm*	*Mm*	*Mm*	*Mm*	*Mm*	*Mm*	*Mm*	*Mm*	*Mm*	*Mm*
1	0.08	1.8	4.1	7.0	11.3	19.7	27.7	31.1	31.4	29.0	22.1	10.3	0.15
2	.10	1.9	4.2	7.1	11.5	19.9	27.9	31.2	31.9	29.3	22.5	10.5	.20
3	.09	1.8	4.1	7.0	11.4	19.8	27.8	31.1	31.7	29.1	22.2	10.4	.19
4	.10	1.9	4.3	7.2	11.6	20.0	27.9	31.3	31.9	29.4	22.7	10.6	.21
5	.10	1.9	4.2	7.1	11.5	19.8	27.9	31.2	32.0	29.2	22.6	10.5	.20
6	.10	1.9	4.2	7.2	11.5	19.9	27.8	31.2	31.9	29.3	22.5	10.5	.20

These findings are in accord with other reports of temperature relations of *Pythium ultimum*. Jones (*23*) states that *P. ultimum* isolated from decayed tubers of potato was found to have a minumum temperature of approximately 4° C., an optimum of 28°, and a maximum of 40°. Likewise Wager (*40*) has found the minimum temperature of *P. ultimum* isolated from *Arachis hypogea*, *Carica papaya*, *Ipomoea batatas*, *Papaver nudicaule*, *Rheum rhaponticum*, and *Striga lutea* to be rather low, approximately 5°. The optimum was 30° with the exception of an isolate each from *C. papaya* and *S. lutea*, in which case the optimum was 35°. The maximum was 40° with the exception of the two isolates having a higher optimum. These latter cultures were found to have a correspondingly higher maximum temperature value. According to Pirone et al (*32*) the minimum temperature of *P. ultimum* isolated from spinach is somewhat lower than 9°, the optimum approximately 30°, the maximum 38°. Alexander, Young, and Kiger (*1*) report the optimum temperature of *P. ultimum* from tomato to be from 25° to 30°, the minimum about 10°. No maximum value is given.

Since temperature-growth relations are considered a specific feature, it seems quite probable that the cucurbit isolates are similar. The temperature relations are presented here in support of the identification of the cucurbit organisms as *Pythium ultimum*, and indicate physiological as well as morphological similarities.

PATHOGENICITY

The method of inoculation was essentially that described recently by Tompkins and Tucker (*38*). Inoculum was prepared by growing pure cultures of the three isolates of *Pythium ultimum* on malt-extract agar in Petri dishes for 48 hours at room temperature. Healthy Zucchini and Mammoth Summer Crookneck pumpkin and watermelon fruits were washed in running tap water, rinsed in distilled water and dried. A small square of inoculum was placed on the uninjured

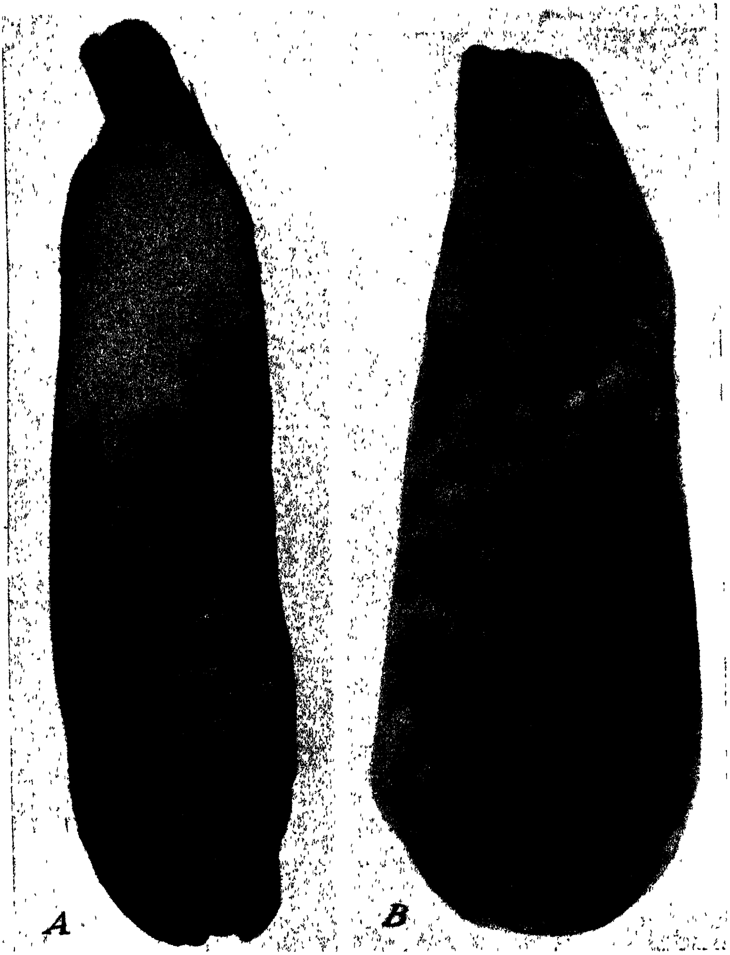

FIGURE 3.—Artificial infection of Zucchini pumpkin fruit: *A*, Longitudinal section of a diseased fruit, showing the soft, water-soaked tissues produced without wounding, 6 days after inoculation with a 3-day-old culture of *Pythium ultimum*. *B*, section from the same fruit, showing the type of aerial mycelium that develops in a moist chamber and a large water-soaked lesion free from mycelium. This fruit was completely rotted within 10 days.

epidermis of the pumpkin fruits, and the fungus was inserted in small scalpel wounds through the rind of the watermelon fruits. The inoculum was kept moist with absorbent cotton under an inverted preparation dish. For controls, fruits were handled in the same manner except that sterile agar was substituted for the inoculum. All inoculations were made at room temperatures (20° to 23 ° C.).

Of 12 Zucchini pumpkin fruits inoculated, 3 were infected after 3 days as indicated by the soft, sunken, water-soaked lesions which measured approximately 7 cm in diameter and slightly more internally (fig. 3, *A*). After 4 days, four additional fruits were infected, with lesions averaging 10 cm in diameter. All the remaining fruits which showed infection after 5 to 7 days had lesions with an average diameter of 12 cm, except one which was completely rotted. These artificially induced lesions were identical in color and consistency with those on naturally infected fruits. The six noninoculated control fruits remained healthy. The fungus was reisolated from each infected fruit, and subsequently 10 healthy fruits were inoculated with the reisolated fungus. All inoculated fruits were diseased at the end of 5 days. Superficial growth of mycelium was not observed unless infected fruits were placed in moist chambers (fig. 3, *B*).

Comparable results were obtained in tests with Mammoth Summer Crookneck pumpkin fruits. Of nine fruits inoculated, eight were infected in 3 to 4 days. Of four Klondyke and four Cooper watermelons inoculated, all were infected within 6 days and totally decayed within 10 days. Inoculations with the reisolates from Mammoth Summer Crookneck pumpkin and watermelon fruits were successful.

Isolates from each of the three hosts proved pathogenic to the other two. However, infection of watermelon fruits with the two isolates from pumpkin fruits occurred only after wounding.

No infection occurred when cultures of the fungus on sterilized cracked wheat were introduced into autoclaved soil in 6-inch pots containing Zucchini and Mammoth Summer Crookneck pumpkin and watermelon seedlings; however, when the inoculum was placed in the soil immediately after seeding, damping-off occurred. About 90 percent of the plants were killed in the preemergence stage, and the remainder collapsed soon after emergence.

Isolations of *Pythium ultimum* from cucurbit fruits, roots of alfalfa, spinach, and tobacco, and the Baarn culture (host unknown) caused damping-off of tomato seedings; 50 tomato seeds were sown in infested soil in each of five 6-inch pots and survival counts were made 15 days later. The following results were obtained:

Source of isolate	*Seedlings surviving after 15 days*
Baarn (host unknown)	3
Citrullus vulgaris	10
Cucurbita pepo	2
Do	2
Do	2
Medicago sativa	3
Nicotiana tabacum	3
Spinacia oleracea	23
Check (not inoculated)	220

All isolates behaved similarly, including the nonoospore-producing isolates from cucurbit fruits. The damping-off symptoms were identical with those reported for *Pythium ultimum* on tomato and other seedlings by other investigators.

From these tests, it is concluded that *Pythium ultimum* is the cause of the fruit rot described here, and that it is an aggressive parasite capable of direct penetration of pumpkin but not of watermelon tissues.

EXPERIMENTAL HOST RANGE

Various hosts were inoculated by the no-wound technique described for the pathogenicity tests. Reisolations were made from all diseased specimens. The reisolate of the fungus from a particular host was then tested by inoculation into healthy specimens of that host.

The experimental host range of the three isolates of *Pythium ultimum*, as determined by artificial inoculations made simultaneously in the laboratory, is quite limited. Fruits of green and ripe tomato (*Lycopersicum esculentum* Mill. var. *vulgare* Bailey) var. Stone, bean (*Phaesolus vulgaris L.*), eggplant (*Solanum melongena* L. var. *esculentum* Nees), and field pumpkin (*Cucurbita pepo* L.) proved susceptible. Lesions which developed after 2 to 5 days were soft, water-soaked, and slightly sunken in appearance except those on eggplant which were warm sepia (*36*) in color.

No infection was obtained on unwounded fruits of apple (*Malus sylvestris* Mill.) var. Newtown Pippin, bell or green sweet pepper (*Capsicum annuum* L. var. *grossum* Sendt.), squash (*Cucurbita maxima* Duchesne) var. Banana, pumpkin (*C. pepo* var. *condensa*) var. Early White Bush Scallop and Pie, watermelon (*Citrullus vulgaris*), Casaba and Honeydew melons (*Cucumis melo* L. var. *inodorus* Naud.), Persian melon (*C. melo* var. *reticulatus* Naud.), and cucumber (*C. sativus*); on unwounded roots of turnip (*Brassica rapa* L.) var. Purple Top White Globe, rutabaga (*B. campestris* L. var. *napo-brassica* DC.), carrot (*Daucus carota* L. var. *sativa* DC.), and parsnip (*Pastinaca sativa* L.); and on unwounded potato tubers (*Solanum tuberosum* L.) var. Russet Burbank.

All fruits, roots, and tubers which had reacted negatively to inoculation without wounding, as noted in the preceding paragraph, were readily infected upon wounding. In addition, fruits of lemon (*Citrus limonia* Osbeck) and sweet orange (*C. sinensis* Osbeck), storage organs of Bermuda onion (*Allium cepa* L.) and sweetpotato proved susceptible when wounded. Soft, slightly sunken, water-soaked lesions appeared on fruits and roots within 3 days and on potato tubers in 4 days. Internally, infected tubers had a blackened cortex; the parenchyma was unchanged in color and soft in consistency.

SUMMARY

A soft rot of Zucchini and Mammoth Summer Crookneck pumpkin and watermelon fruits, prevalent in California, is described.

The disease occurs when fruits of any size or age are in contact with wet soil and is favored by cool weather.

Symptoms of the disease consist of soft, sunken, water-soaked lesions which quickly enlarge, causing the underlying tissues to collapse. Invaded fruits may be completely rotted within 6 to 10 days after infection occurs.

The causal organism has been identified as *Pythium ultimum* Trow. Certain cucurbit isolates did not produce oospores, but were indistinguishable from normal cultures by growth or pathogenic characters.

Reproductive bodies resembling oogonia developed in cultures of nonoospore-producing isolates. It is suggested that the absence of oospore formation is the result of failure of the organism to develop antheridia.

The temperature relations of the organism were determined. The minimum was 4° C., optimum 25° to 28°, maximum 40°. All cucurbit isolates, including the two nonoospore-producing cultures, isolates from roots of alfalfa, tobacco, and spinach and a culture from an unknown host, had identical temperature relations.

Infection of healthy pumpkin and watermelon fruits was obtained by inoculation in the laboratory. However, wounding of watermelon fruits was necessary in order to induce decay.

All isolates caused damping-off of pumpkin, watermelon, and tomato seedlings during the preemergence and small-seedling stages of growth. Older plants were resistant.

Green and ripe tomatoes, eggplant, beans, and field pumpkin proved susceptible to infection, without wounding, under laboratory conditions. When wounded, the following hosts proved susceptible: Apple, bell pepper, squash, pumpkin (two varieties), watermelon, Casaba, Honeydew, and Persian melons, cucumber, turnip, rutabaga, carrot, parsnip, potato, lemon, sweet orange, onion, and sweetpotato.

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THE FOLIAR DIAGNOSIS OF ZEA MAYS SUBJECTED TO DIFFERENTIAL FERTILIZER TREATMENT [1]

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INTRODUCTION

In the method of foliar diagnosis (*1*, *5*)[2] the development and yield of a plant is related to the series of chemical transformations taking place in the synthetic laboratory of the plant, viz the leaf. The experimental facts established by the method are proof that this basis of reference is more reliable and in addition, supplies much more information concerning the factors governing the development and yield of a plant than does the traditional method of relating ultimate yields solely to the fertilizers applied. Since it requires only the collection of a few leaves it is not only simpler and more practical, but is a much more sensitive method of control than that requiring the analysis of the entire plant (*3*).

Foliar diagnosis makes it possible to study the variations in the mineral nutrition of the plant resulting from very diverse factors—physical, chemical, and biological. The evidence obtained thus far (*1*, *5*, *6*, *7*) indicates that the composition of a leaf is modified both quantitatively and qualitatively with respect to nitrogen, phosphoric acid, and potash by factors hitherto little suspected.

There are valid grounds for believing that sense can be made of the chaotic mass of information in agronomical literature by the application of the method to problems of fertilizer effects that now appear paradoxical.

The experimental facts upon which the validity of the method is based consist in essence of the following findings:

(1) Two morphologically homologous leaves of the same species and variety are the seat of identical physiological processes when the medium (soil and meteorological factors) is identical, and of different physiological processes when the mediums are different (*1*, *5*).

(2) Whenever a fertilizer element (whether N or P or K) is effective, as determined by response to that element, the response is invariably associated with an increase of that element in the dried material of the leaf (*1*, *5*, *6*, *7*, *8*).

(3) The foliar diagnoses of plants of the same species growing on the same homogenous medium (soil and meteorological factors) but receiving different fertilizer treatments are correlated with their development and also with the nature of the fertilizer applied (*1*, *7*).

It is emphasized that the results by the method of foliar diagnosis are comparative just as the results of the entire analysis of plants are comparative (*3*). No physiological significance, therefore, can be attached to the foliar diagnosis of any one fertilizer treatment (plot) considered alone.

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[2] Italic numbers in parentheses refer to Literature Cited, p. 491.

The results are expressed on the only suitable basis for this type of investigation, viz, on the basis of the dry weight of the leaves. All results by means of which the method has been established are on the basis of the percentage of the element in the dried foliage.

In the investigations on foliar diagnosis carried out by the present writers thus far the influence of calcium and magnesium on the N-P-K nutrition has not been examined, although the desirability for such knowledge has been pointed out (*8*). Since the foliar diagnosis, with respect to Ca, of plants sprayed with calcium-containing sprays would be worthless, *Zea mays* growing on certain plots of the Jordan fertility experiment of the Pennsylvania Agricultural Experiment Station was used for the present studies. An unfertilized plot, not available in the earlier studies of the method, was also included. This paper reports the results of these studies.

Ranking in age second only to the Rothamsted experimental plots, the Jordan plots laid out in 1881 afford interesting experimental material for the development of laws, principles, or axioms of plant nutrition under field conditions.

In long-continued field experiments in which a given plot receives year after year the same fertilizer, any disequilibrium becomes progressively greater and greater with time. Trials of a few years only may frequently not show this disequilibrium.

MATERIALS AND METHODS

The Jordan fertility plots (*2*) consist of 144 plots of one-eighth acre each, arranged in four tiers consisting of 36 plots. On each tier a 4-year rotation is followed in which corn, oats, wheat, and hay (mixed clover and timothy) are each grown for 1 year, the fertilizer treatments being applied to the corn and the wheat.

A detailed description of the method of leaf sampling was given in an earlier publication (*5*). The third leaf from the base of a stalk of corn was taken on July 6, July 21, August 8, and August 25, 1936, from all of the plants along a row lengthwise of each plot, omitting plants that deviated considerably from the norm. Successive rows were then sampled on each of the sampling dates indicated.

The plot numbers, treatments, and yields of the plots examined are given in table 1.

TABLE 1.—*Plot, treatments, and yields in tier 1*

Plot No.	Treatment	N, P_2O_5, and K_2O equivalent applied to each plot	Symbol and ratio	Yield per plot	
				Grain	Stover
		Pounds		*Pounds*	*Pounds*
1	Nothing	0		165.4	117.1
2	Dried blood	3	N	265.6	143.4
3	Superphosphate	6	P	371.2	220.1
4	Muriate of potash	12.5	K	298.4	181.7
5	Dried blood Superphosphate	3 6	NP, 6:12:0	376.7	195.9
6	Dried blood Muriate of potash	3 12.5	NK, 6:0:25	263.5	171.7
7	Superphosphate Muriate of potash	6 12.5	PK, 0:6:25	497.4	254.5
9	Dried blood Superphosphate Muriate of potash	3 6 12.5	NPK, 6:12:25	520.2	214.0

METEOROLOGICAL CONDITIONS DURING THE 1936 GROWING SEASON

As a matter of record the meteorological conditions for the month preceding the sampling, and for the intervals between sampling are given in table 2.

TABLE 2.—*Rainfall and average daily mean temperatures during the month preceding sampling and during the intervals between sampling*

Period	Rainfall	Mean daily temperature	Period	Rainfall	Mean daily temperature
	Inches	° *F.*		*Inches*	° *F.*
June 6–July 5	5.04	66.7	July 22–Aug. 8	3.38	70.6
July 6–July 21	.51	75.3	Aug. 9–Aug. 25	2.48	74.4

PRESENTATION OF RESULTS

The results of the analysis of the third leaf from the base are shown in table 3 expressed as (1) percentages of nitrogen, phosphoric acid, potash, lime, and magnesia in the dried foliage, (2) milligram equivalents, and (3) the composition of the NPK unit (*5*).

THE DERIVATION OF THE NPK UNIT

Two concepts, viz, that of quantity and that of quality of the mineral constituents are inherent in the expression of the analysis in terms of the dried weight of the leaves. The quantity or intensity of nutrition of the selected leaves consists of the sum $(N+P_2O_5+K_2O)$ of the dominant fertilizer entities at the moment of sampling, the data for which are shown in column 7. The quality of nutrition is the ratio of these entities to each other also at the moment of sampling. Since this factor is dependent upon and related to the chemical reactions involved in the leaf, it is necessarily expressed in milligram-equivalent values.

Let M_x, M_y, M_z be the percentages of nitrogen, phosphoric acid, and potash in the leaf at the moment of sampling. Then, expressing in milligram-equivalents:

$$E_x=1{,}000\frac{M_x}{N};\ E_y=1{,}000\frac{M_y}{\frac{1}{6}(P_2O_5)};\ E_z=1{,}000\frac{M_z}{\frac{1}{2}(K_2O)}$$

Putting $S=E_x+E_y+E_z$ and dividing by S, we get $1=\frac{E_x}{S}+\frac{E_y}{S}+\frac{E_z}{S}$. The last three terms represent respectively in milligram-equivalent values the proportional parts of N, P_2O_5, and K_2O in a unit quantity of these elements. To avoid fractional values the terms are multiplied by 100. If the respective values obtained be denoted by X, Y, Z then $X+Y+Z=100$. This is the NPK unit shown in the last three columns of table 3.

TABLE 3.—*The percentages of N, P_2O_5, K_2O, CaO, and MgO in the dried foliage, milligram equivalents, and the composition of the NPK unit for plants grown under different fertilizer treatment in tier 1*

Date	Mineral content of dried foliage						Milligram equivalents				Composition of the NPK unit		
	N—(M_x)	P_2O_5—(M_y)	K_2O—(M_z)	CaO—	MgO—	$N+P_2O_5+K_2O$—$M_x+M_y+M_z$ (s)	N—(E_x)	P_2O_5—(E_y)	K_2O—(E_z)	$E_x+E_y+E_z$—S	X—$\left(100\frac{E_x}{S}\right)$	Y—$\left(100\frac{E_y}{S}\right)$	Z—$\left(100\frac{E_z}{S}\right)$
					NOTHING (PLOT NO 1)								
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*							
July 6	2.960	0.307	2.387	2.308	0.934	5.654	211.344	12.986	50.843	275.173	76.804	4.719	18.477
July 21	2.210	.305	2.515	2.087	.936	5.030	157.794	12.901	53.569	224.264	70.361	5.753	23.886
Aug. 8	1.800	.230	1.841	1.605	1.039	3.871	128.520	9.729	39.213	177.462	72.421	5.482	22.097
Aug. 25	2.010	.316	1.608	1.999	1.216	3.934	143.514	13.367	34.250	191.131	75.089	6.993	17.920
					N (PLOT NO 2)								
July 6	3.310	0.311	2.248	2.058	1.177	5.809	236.334	13.155	47.882	297.371	79.474	4.424	16.102
July 21	2.420	.330	2.104	2.435	1.448	4.854	172.788	13.959	44.815	231.562	74.618	6.028	19.353
Aug. 8	2.090	.260	1.453	2.328	1.376	3.803	149.226	10.998	30.949	191.173	78.059	5.753	16.189
Aug. 25	2.340	.348	1.481	2.222	1.159	4.169	167.076	14.720	31.545	213.341	78.314	6.899	14.786
					P (PLOT NO. 3)								
July 6	3.210	0.656	1.019	3.131	1.901	4.888	229.194	27.749	21.705	278.648	82.252	9.958	7.789
July 21	2.260	.841	.756	3.744	2.462	3.857	161.364	35.574	16.103	213.041	75.743	16.698	7.559
Aug. 8	2.060	.602	.794	3.348	1.847	3.456	147.084	25.465	16.912	189.461	77.633	13.441	8.926
Aug. 25	2.100	.678	.911	2.937	1.792	3.689	149.940	28.679	19.404	198.023	75.718	14.483	9.799
					K (PLOT NO 4)								
July 6	2.980	0.301	3.984	1.690	0.815	7.265	212.772	12.732	84.859	310.363	68.556	4.102	27.342
July 21	2.280	.263	4.000	1.887	1.112	6.543	162.792	11.125	85.200	259.117	62.826	4.293	32.881
Aug. 8	2.320	.270	3.901	1.689	.905	6.491	165.648	11.421	83.091	260.160	63.672	4.390	31.938
Aug. 25	2.020	.392	2.480	1.491	.742	4.892	144.228	16.582	52.824	213.634	67.512	7.762	24.726

NP (PLOT NO 5)

July 6	3.210	0.928	0.724	3.410	2.114	4.862	229.194	39.254	15.421	283.869	80.739	13.828	5.432
July 21	2.410	.692	.813	3.322	2.516	3.915	172.074	29.272	17.317	218.663	78.694	13.387	7.919
Aug. 8	2.360	.690	.813	3.322	2.027	3.863	168.504	29.187	17.317	215.008	78.391	13.575	8.054
Aug. 25	2.370	.768	.775	2.984	2.281	3.913	169.218	32.486	16.507	218.211	77.548	14.887	7.565

NK (PLOT NO. 6)

July 6	3.040	0.336	4.031	1.543	0.634	7.407	217.056	14.213	85.860	317.129	68.444	4.482	27.074
July 21	2.390	.280	4.209	1.507	.833	6.879	170.646	11.844	89.652	272.142	62.705	4.352	32.943
Aug. 8	2.000	.262	3.081	1.628	.905	5.343	142.900	11.083	65.025	219.508	65.055	5.049	29.896
Aug. 25	2.380	.376	2.763	1.309	[illegible]	5.519	169.932	15.905	58.852	244.689	69.448	6.500	24.052

PK (PLOT NO 7)

July 6	3.040	0.440	3.565	1.902	0.605	7.045	217.056	18.612	75.934	311.602	69.658	5.973	24.369
July 21	2.280	.476	3.508	2.221	.869	6.264	162.792	20.135	74.720	257.647	63.184	7.815	29.001
Aug. 8	1.960	.420	2.887	2.283	.688	5.267	139.944	17.766	61.493	219.203	63.842	8.105	28.053
Aug. 25	2.080	.534	2.665	1.917	.507	5.269	148.512	22.588	56.551	227.651	65.237	9.922	24.841

NPK (PLOT NO 9)

July 6	3.03	0.438	3.674	1.720	0.606	7.506	216.342	18.527	78.256	313.125	69.091	5.917	24.992
July 21	2.460	.454	3.766	1.887	.707	6.680	175.644	19.204	80.216	375.064	63.856	6.982	29.163
Aug. 8	2.620	.564	2.992	1.842	.362	6.176	187.068	23.857	63.729	274.654	68.110	8.686	23.203
Aug. 25	2.540	.626	2.879	1.705	.271	6.045	181.356	26.479	61.323	269.158	67.379	9.838	22.783

DISCUSSION AND INTERPRETATION OF RESULTS

In the discussion which follows, the treatment symbol followed by the number of the plot is a brief method of expressing the content of N, P_2O_5, or K_2O in the third leaf of plants growing on the particular plot with the treatment represented. For example, "N in NP (No. 5)" means the content of N in the third leaves of plants growing on the plot fertilized with NP, which is plot No. 5.

NITROGEN

At the first sampling the differences in the nitrogen content of the leaves of plants growing on check (No. 1), K(No. 4), NK(No. 6), PK(No. 7), and NPK(No. 9) plots were small (0.08 percent or less), with no difference between those of P(No. 3) and NP(No. 5). At this date the content of N was highest in N(No. 2), followed by P(No. 3) and NP(No. 5).

With advancing age, the leaves from the plots receiving nitrogen became more and more differentiated, in their N content, from those on plots receiving none. This difference shows that the nitrogen of dried blood, slowly available during the first period, was now becoming more available.

The nitrogen content did not decrease regularly with the advancing age of the leaves of plants growing on any of the plots considered in this paper. Accumulation of nitrogen took place alike in plots treated with nitrogen as commercial fertilizer (dried blood) and in those not receiving nitrogen. Thus accumulation took place in the last period in check, N, P, NK, and PK and during the second period in K and NPK, showing slow and poor utilization of nitrogen even by plants which received nitrogen applications in the form of dried blood and which had never received lime amendments.

At two sampling dates only, July 21 and August 25, are the leaves of plants growing on plots that received nitrogen applications all higher in nitrogen than those on plants that did not receive any. At these dates there is a relationship, consequently, between the content of nitrogen in the leaf and in the fertilizer, but none between nitrogen in the leaf and the yield, nor between the presence of nitrogen in the fertilizer and the yield, except when accompanied by phosphoric acid and potash.

Graphed in the manner previously described (*4*), marked disequilibrium between nitrogen and phosphoric acid of the leaves of plants from all treatments is shown (fig. 1). At the first sampling date the phosphoric acid content of the leaf is too low in relation to the nitrogen in all treatments except P and NP. In the latter and also in PK after the first period, the nitrogen is too low in relation to the phosphoric acid throughout the whole period from July 6 to August 25. After July 21, no uniformity in the relations between nitrogen and phosphoric acid of the leaf is shown. Nitrogen is much too high in relation to phosphoric acid in both check and N throughout the whole period and also in K up to August 8, when the relationship is reversed. In NPK the tendency is toward nutritional equilibrium between N and P_2O_5 up to the beginning of the third period, after which the nitrogen becomes too low in relation to the P_2O_5.

PHOSPHORIC ACID

Phosphoric acid does not decrease progressively with the advancing age of the leaf. In the leaves of check, N, K, NK, PK, and NPK a maximum occurs at the last sampling, whereas in P and NP the maximum occurs on July 21 and July 6, respectively.

The differences in the foliar diagnosis caused by the presence of phosphoric acid in the fertilizer, although small absolutely, are very striking relatively. Thus a variation from 0.3 percent in K to 0.6 percent in P is a variation of 100 percent.

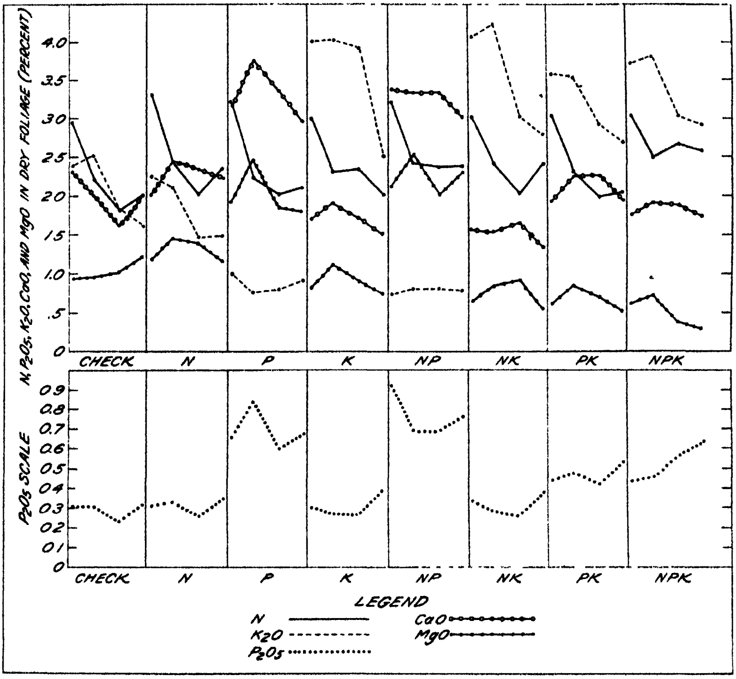

FIGURE 1.—Changes during the growth cycle in the content of N, P_2O_5, K_2O, CaO, and MgO in the third leaf resulting from different treatments. Percentage values in terms of the dried foliage as ordinates and dates of sampling as abscissae.

Phosphoric acid is very much higher throughout the whole period in the leaves of plants which received superphosphate additions. Thus, on July 6 the phosphoric acid content of the selected leaves from plots which received superphosphate applications is 0.438 percent or more; whereas in those which did not receive phosphate fertilizer the content of P_2O_5 is 0.336 percent or less.

Furthermore, the leaf content with respect to P_2O_5 is highest throughout the whole period in the four plots which have the highest yields—P, NP, PK, and NPK. But the highest yield does not correspond to the P_2O_5 graph which is the highest—viz, the NP, but to NPK in which the P_2O_5 graph is the next to the lowest of these four highest yielding plots. Accordingly, the additional supplement of P_2O_5 absorbed by the leaves from P and NP has not been utilized in growth—an example of "luxuskonsumption" of phosphorus.

A relationship, therefore, exists between phosphoric acid in the leaf and phosphoric acid in the fertilizer, between phosphoric acid in the leaf and the yield, and between phosphoric acid in the fertilizer and the yield.

POTASH

The potash content of the leaves of plants growing on the plots that received potash fertilizer is very much higher than in those that did not. At the first sampling, on July 6, the content of K_2O was 3.56 percent or more in the leaves of plants that received potash applications and only 2.38 percent or less in those that did not.

The K_2O graphs of P and NP, two of the four highest yielding plots, are lower than any other of the treatments, the causes for which will be considered later. Furthermore, the leaf content in K_2O of NK, the second lowest yielding plot, is on the first two sampling dates the highest of any of the treatments.

There is consequently no relationship between the presence of potash in the fertilizer and the content of potash in the leaf, and there is no relationship between the variation, however great, of the potash in the leaf and the variations in the yield. There is, moreover, no relationship between the presence of potash in the fertilizer and the yield; thus, potash increased the yields when applied alone or with phosphate, but not when applied with nitrogen.

LIME AND MAGNESIA

With the exception of NP, the changes in direction of the graphs for MgO with lapse of time are similar to those of the respective graphs for CaO.

In leaves of plants developing normally a progressive increase occurs in the CaO and MgO content of the leaf with advancing age. But in check, P, K, NP, NK, and NPK, the CaO content was lower at the last sampling on August 25 than at the first sampling on July 6 and, moreover, the increase in CaO with the advancing age of the leaf was very small in the remaining treatments—N and PK. It should be kept in mind that none of these plots has been limed since 1881.

The effect of superphosphate with and without potash applications on the absorption of calcium and magnesia is strikingly shown in table 4, in which the results for CaO and MgO are given as the mean of the results in each case of the four samplings expressed as a percentage of the dried foliage.

TABLE 4.—*The effect of superphosphate with and without muriate of potash on the absorption of calcium and magnesium*

Plot No	Treatment	CaO	MgO	Plot No.	Treatment	CaO	MgO
		Percent	*Percent*			*Percent*	*Percent*
3	P	3.29	2.00	2	N	2.26	1.29
5	NP	3.26	2.23	4	K	1.69	.89
7	PK	2.08	.66	7	NK	1.50	.72
9	NPK	1.79	.49	23	CaO	3.55	1.18
1	Nothing	2.60	1.03				

The CaO and MgO contents of the leaves of P and NP, which received superphosphate without potash additions, are relatively high; the CaO content is nearly as high as in the leaves from the plot which received dressings of lime only without fertilizer, whereas the MgO content is actually higher in P and NP than in the lime plot. On the other hand, in PK and NPK, which received both superphosphate and potash applications, the CaO and MgO contents of the leaves are very much lower. Confirmation of this depressing effect of muriate of potash on the absorption of CaO and MgO is shown by a comparison of the results of the CaO and MgO contents of the leaves of K and NK with those of check and N.

Nevertheless, the reduction in the CaO and MgO content of the leaves of NPK and PK below the values in P and NP has had no adverse effect on the yields of NPK and PK. It would appear, consequently, that calcium and magnesium tend to replace potassium when this element is deficient. The functions of potassium in which it may be replaced by calcium and magnesium can be only conjectured, but are probably connected with controlling the hydrogen-ion concentration of the sap.

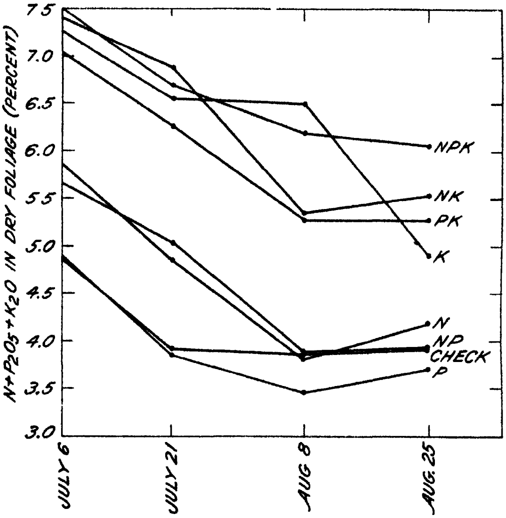

FIGURE 2.—Intensities of nutrition of the leaves at four dates of sampling.

THE INTENSITIES OF NUTRITION

The magnitudes representing the intensities of nutrition (*5*) of the chosen leaves on the respective sampling dates are given in column 7, table 2, and are shown graphically in figure 2.

Two groups are recognized, check, N, P, and NP, having low intensities of nutrition, and K, NK, PK, and NPK, with relatively high intensities of nutrition. The addition of potash, then, invariably resulted in an increase in the intensity of nutrition of the leaf as compared with that of those which did not receive potash treatment; but, as already pointed out (*5*), there is not necessarily any relation between intensity taken alone and the yields. A fertilizer may intervene to cause (1) a change in the intensity or (2) a change in the NPK equilibrium, or a change in (1) and (2) simultaneously. It is to be noted that NPK has the highest mean intensity and the highest yield of the plots here considered. The intensities resulting from the other treatments must consequently be regarded as below the optimum in satisfying the mineral requirements with respect to N, P, and K.

COMPOSITION OF THE NPK UNITS

The composition of the NPK units at the respective sampling dates is shown in the last three columns of table 3. The diagrammatic representation of this unit has been given previously (*5*). The NPK unit represents the equilibrium between nitrogen, phosphoric acid, and potash in the chosen leaves at the moment of sampling.

It has been shown (*5*) that this unit is independent of the intensity factor of nutrition ((*8*) table 3) and consequently is a magnitude representing the quality factor of nutrition as distinguished from the intensity or quantity factor. These two mangitudes representing the quality and quantity factors, respectively, in the selected leaf although abstractly distinct are inseparably connected with the physiological processes of the leaf and are, therefore, to be considered simultaneously.

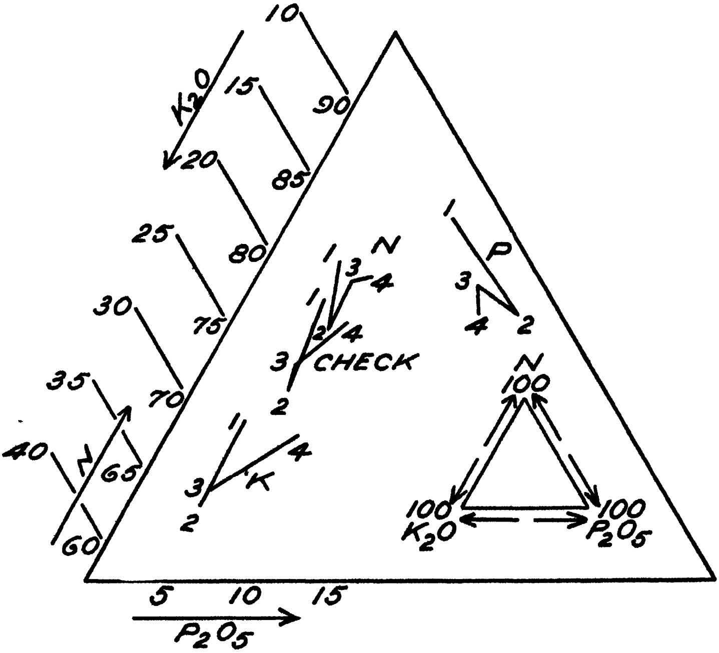

FIGURE 3.—Changes during the growth cycle in the N–P_2O_5–K_2O equilibrium in the third leaf from plants growing on N, P, K, and nothing (check) plots. The numerals indicate the coordinate point of the NPK unit at the respective sampling date.

It must be borne in mind further that the effect of a fertilizer is the resultant of (1) the effect of the fertilizer itself, and (2) of the medium (soil and meteorological factors) on the N–P_2O_5–K_2O equilibrium.

THE COURSE OF NUTRITION WITH RESPECT TO NPK

The detailed diagrams showing the course of nutrition with respect to NPK during the period of sampling are given in figures 3, 4, and 5. The points represented by the numerals are the coordinate points at the four successive samplings. It is not necessary to discuss these graphs in detail, for the eye can follow the course of nutrition resulting from the different treatments by following the lines from one sampling date to the next.

The reader should keep in mind that the changes in the composition of the NPK unit of any treatment with time corresponds with the changes in the N–P_2O_5–K_2O equilibrium with the advancing age of the leaf (*5*) as the result of the influence of the medium (soil and climate).

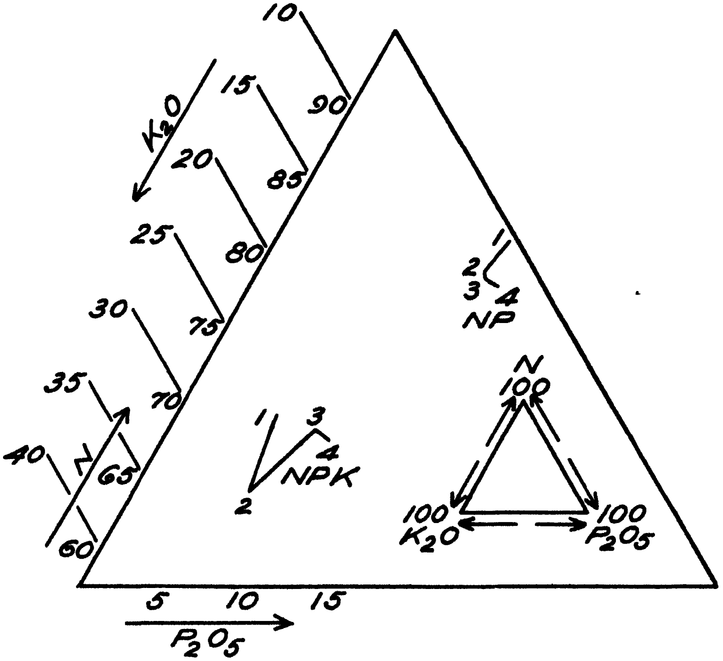

FIGURE 4.—Changes during the growth cycle in the N–P_2O_5–K_2O equilibrium in the third leaf from plants growing on the NP and NPK plots. The numerals indicate the coordinate point of the NPK unit at the respective dates of sampling.

The graphs show the following characteristics: Throughout the earlier period (1–2) potash increases at the expense of the nitrogen, and in the two treatments (NP, NK) at the expense of the P_2O_5 also During the remainder of the growth period (2–4), nitrogen increases chiefly at the expense of the potash, except in two treatments (P and NP).

These facts reflect the slow availability of dried blood on unlimed plots. Comparison of the graphs of the treated plots with that of the check plot shows that the addition of nitrogen in the form of dried blood has been unable to overcome, during the earlier period, the declining tendency of the medium (soil and meterological factors) with respect to nitrogen supply. The reversal in direction after

July 21 of the graph for the check plot shows that the unfavorable influence of the medium with respect to nitrogen supply is arrested and becomes more favorable as the season progresses.

THE MEAN NPK UNIT

The center of gravity of each of these diagrams represents the mean NPK unit of the leaf of the rank selected. The mean NPK units of the leaves of plants growing on the respective plots, therefore, can be indicated on the triangle by means of a point, as shown in figure 6.

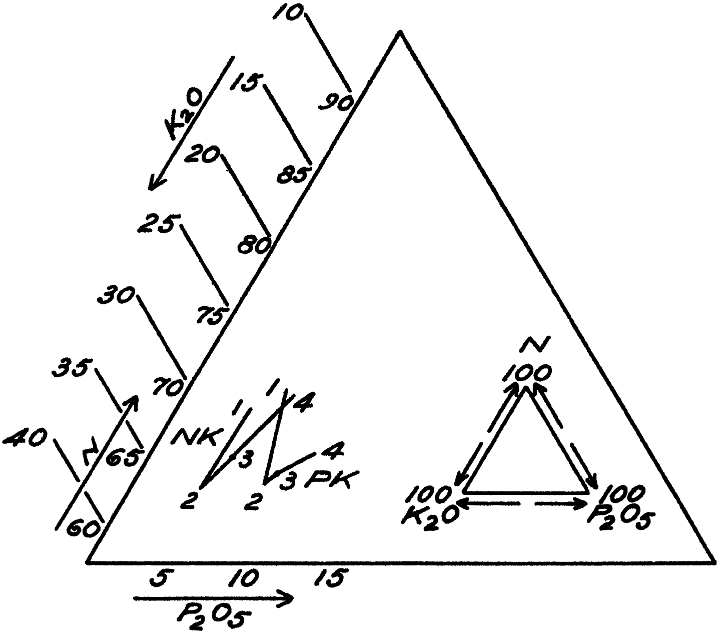

FIGURE 5.—Changes during the growth cycle in the N–P_2O_5–K_2O equilibrium in the third leaf from plants growing on the NK and PK plots. The numerals indicate the coordinate point of the NPK unit at the respective dates of sampling.

Table 5 lists the mean NPK units and the mean intensity of nutrition together with the yields of grain and stover.

TABLE 5.—*The relation of the composition of the NPK unit and intensity to yields*

Group No.	Plot No.	Treatment	Mean intensity of nutrition	Mean NPK-unit	Yields per plot	
					Grain	Stover
					Pounds	*Pounds*
1	1	Check	4.62	73.67:5.74:20.59	165.6	117.1
	6	NK	6.29	66.41:5.10:28.49	263.5	171.7
	2	N	4.67	77.62:5.77:16.61	265.6	143.4
	4	K	6.29	65.64:5.14:29.22	298.4	181.7
2	3	P	3.97	77.84:13.64:8.52	371.2	220.1
	5	NP	4.14	78.84:13.92:7.24	367.7	195.9
3	7	PK	5.96	65.48:7.95:26.57	497.4	254.5
	9	NPK	6.60	67.11:7.85:25.03	520.2	214.0

THE CLASSIFICATION INTO THREE GROUPS ACCORDING TO THE POSITION ON THE TRIANGLE OF THE MEAN NPK UNITS

The treatments fall into three groups according to their position on the triangle.

Group 1: Check, N, K, and NK. Poorly nourished plants having the lowest yields from any of the plots, and characterized by a position on the triangle further to the left—i. e., further removed from the apex representing $P_2O_5=100$ percent.

Group 2: P and NP. Better nourished, with yields higher than those of group 1 and characterized by a position further to the right—toward the apex representing $P_2O_5=100$ percent, and relatively high up toward the apex $N=100$ percent.

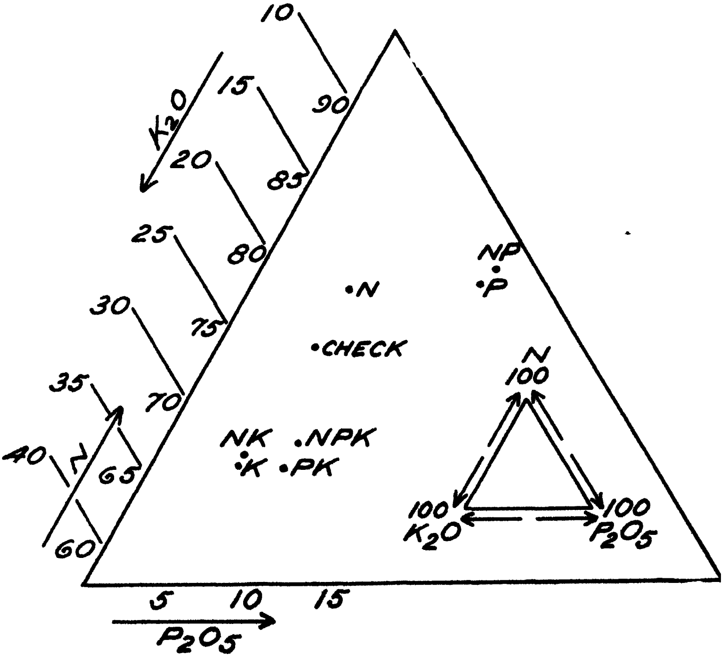

FIGURE 6.—Relative positions of the mean NPK units of the third leaf from plants growing on the plots indicated. The mean NPK unit represents the center of gravity of the respective graphs of figures 3 to 5.

Group 3: NPK and PK. Much better nourished plants having the highest yields from any of the plots considered in this paper. These two plots are characterized by a position further to the right of the lowest yielding group 1, and to the left of the intermediate yielding group 2—i. e., the leaves of plants in this group (3) contain more P_2O_5 in the NPK unit than do those of group 1, and also more K_2O but less P_2O_5 than those of group 2.

As already stated (p. 487) the quality of nutrition must be considered simultaneously with the quantity of nutrition—the intensity factor. In group 1 are to be found plants having relatively low intensities (check, and N) on the one hand, and on the other, those possessing relatively high intensities (K and NK), which are the results, respectively, of a very low and of a relatively high content of K_2O in the NKP unit. Plots in group 2 are characterized by very low intensities of nutrition and those in group 3, the high-yielding group, by high intensities.

SUMMARY

Plants of *Zea mays* growing on unlimed experimental plots treated with different combinations of commercial fertilizers were examined by the method of foliar diagnosis. The plots reported in this paper include eight in tier 1 of the long-continued Jordan fertility experiments of the Pennsylvania Agricultural Experiment Station. The treatment consisted of the following combinations of fertilizers on plots unlimed since the inception of the experiment in 1881: Check, N, P, K, NP, NK, PK, and NPK. The carrier of nitrogen was dried blood; of phosphorus, superphosphate; and of potassium, the chloride, commonly designated as muriate of potash.

Two methods of presenting the results graphically are given, and the indications given by each are discussed.

A relationship exists between the percentage of nitrogen in the dried material of the leaf and in the fertilizer, but none between nitrogen in the leaf and the yield, nor between nitrogen in the fertilizer and the yield except when accompanied by phosphoric acid and potash.

A relationship exists between the percentage of phosphoric acid in the leaf and in the fertilizer; also between phosphoric acid in the leaf and the yield, and between phosphoric acid in the fertilizer and the yield.

No relationship exists between the content of potash in the leaf and in the fertilizer, and none between the variation, however great, of potash in the leaf and the variations in the yield; also there is no relationship between potash in the fertilizer and the yield.

The percentage content of CaO and MgO in the leaves of plants receiving superphosphate without potash (P, NP) was much higher than in those which received potash with the superphosphate (PK, NPK). Moreover the CaO content of the leaves of plants receiving the P and NP treatments differed but little from the CaO content of the leaves from plants which received lime only.

Two concepts peculiar to the method are introduced, viz, that of quantity (intensity of nutrition) and that of quality (physiological ratios of the elements). The latter is conveniently expressed as an NPK unit representing the equilibrium between $N-P_2O_5-K_2O$ in the leaf selected at the moment of sampling. A fertilizer may intervene to affect a change in the (1) quantity or (2) quality or in (1) and (2) simultaneously.

Low intensities of nutrition are associated with low yields (check, P, N, NP) and high intensities with high yields (PK, NPK); but when "luxuskonsumption" of potash occurs, relatively low yields may accompany relatively high intensities of nutrition (K, NK).

The succession of the coordinate points of the NPK unit of the third leaf from the base taken at four sampling periods from plants growing in the differently treated plots are shown in trilinear coordinates.

The treatments fall into three groups according to their position on the triangle—i. e., according to the composition of the respective

NPK units: Group 1, poorly nourished plants (check NK, N, K) characterized by low P_2O_5 in the NPK unit; group 2, better nourished plants (P, NP) with very high P_2O_5, high N, and very low K_2O in the NPK unit; and group 3, best nourished plants (PK, NPK), with values for N, P_2O_5, and K_2O intermediate between those of groups 1 and 2.

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A METHOD OF ESTIMATING THE VOLATILE PRODUCTS LIBERATED FROM STORED FRUIT [1]

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INTRODUCTION

During maturation and senescence, most fruits produce characteristic odorous constituents. Under improper handling and storage practices, certain volatile products of metabolism may form or accumulate in abnormal quantities and become closely associated with definite physiological storage disorders. Common storage scald of apples (*1*) [2] and scald and core break-down of pears (*12*) may have their origin from such causes.

Power and Chesnut (*13, 14*) have shown that the odorous constituents of apples consist chiefly of the amyl esters of formic, acetic, caproic, and caprylic acids, and a considerable proportion of acetaldehyde and ethyl alcohol. Ethylene (*3, 7, 11*), geraniol (*14*), and limonene (*9*) have also been identified as normal volatile products of fruit metabolism. These odorous constituents are all derivatives of three large classifications in structural organic chemistry, namely, the aldehydes, alcohols, and esters of the fatty acid series; the olefines; and the heterogeneous group of essential oils, many of which contain unsaturated hydrocarbons that are usually termed terpenes. These compounds are all chemically reactive and are easily oxidized. They either form addition compounds or undergo polymerization or saponification in the presence of a strong mineral acid. These compounds, therefore, should be capable of absorption in concentrated sulphuric acid and subsequent oxidation with suitable oxidizing reagents.

Many of these volatile components that comprise the odorous constituents of stored fruit are present only in minute amounts. Furthermore, the presence of the various types of individual components may be conditioned by a specific type of physiological metabolism, handling practice, or storage temperature. Acetaldehyde has been identified as one of the more common and more abundant volatile products of fruit metabolism (*12, 13*). This compound can be readily obtained in pure form and is also subject to accurate chemical estimation. For these reasons acetaldehyde has been chosen as representative of the odorous constituents of stored fruit and has been used as a uniform reference standard in the experimental work herein described.

This paper presents a method for the determination of the total oxidizable volatile constituents emanating from stored fruit. The method is based on the absorption of these materials in concentrated sulphuric acid, oxidation with ceric sulphate, and evaluation in terms of ceric sulphate reduced. Several applications of the method are described. This method of measuring volatiles requires the use of

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[2] Italic numbers in parentheses refer to Literature Cited, p. 502.

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simple and inexpensive laboratory equipment, while the necessary reagents are easily prepared and are stable for long intervals of time. The method lends itself to simultaneous routine analysis of a large number of samples and can also be used in conjunction with respiratory measurements of a common lot of fruit. It possesses the accuracy and ease of end-point titration that are characteristic of all iodometric procedures.

REVIEW OF METHODS FOR THE DETECTION OF VOLATILE PRODUCTS FROM FRUITS

Power and Chesnut (*13*) passed the gases emanating from stored Nero apples through a saturated solution of sodium bisulphite. After neutralization and steam distillation of this solution, the presence of acetaldehyde in the distillate was established by the delicate Rimini (dimethylamine) test. Thomas (*19*), using aqueous acid solutions of phloroglucinol, was able to trap and identify acetaldehyde as one of the volatile components liberated from Fen Newtown apples. Volatile substances from ripe apples have been shown by Elmer (*2*) to inhibit normal sprouting of germinating potatoes. He identified the inhibitory substance as ethylene (*3*). Gane (*7*), by absorption in bromine and by preparation of crystalline N. N. diphenylethylene diamine from ethylene dibromide, was able to establish chemically the presence of ethylene among the gaseous products liberated from ripe apples.

Biological assay by means of epinastic responses with Bonny Best tomato plants led Hansen and Hartman (*11*) to surmise that ethylene may be evolved during the ripening of Anjou pears. These investigators were also able to establish the presence of ethylene by chemical means. Oxidation of the gaseous emanations from pears was accomplished by passage through iodine pentoxide at 150° C. Ethylene, as calculated from the iodine liberated, was present in a concentration of approximately 1 part by volume to 70,000 parts of air. Specific solubility of the gaseous emanation in 20-percent mercuric nitrate (2 N in nitric acid) and differential solubility in 87-percent sulphuric acid established the chemical identity of ethylene.

In 1931 Gane (*6*) attempted to concentrate the odorous constituents from the air in a model fruit-storage chamber. Steam distillation of 14 pounds of "drip" from the roof grids gave a clear distillate, the ether extract of which yielded 0.05 gm. of gummy material. In another experiment air from this chamber was drawn through two receivers cooled in solid carbon dioxide and then through two additional collecting tubes immersed in liquid oxygen. Two one-hundredths of a gram of a waxlike material was obtained from the ether extraction of 29 gm. of water in the two former receivers. Odorous substances escaped from the tubes immersed in liquid oxygen immediately upon removal from the lower temperature. Five-tenths of a cubic centimeter of a clear liquid with a very strong odor of apples remained after these receptacles attained room temperature.

Ozone can be determined by its decomposition of aqueous potassium iodide. Gane (*8*) attempted to use this fact as a basis for a method of measuring the volatile emanation from ripe bananas by passing a stream of air carrying a low concentration of ozone over the fruit and estimating the residual ozone after its partial destruction by reaction with the volatiles. He was unable to detect any loss of ozone, however, and intimated that other substances with a carbonyl group may have been present in the air stream and could react with the ozone to form an unstable peroxide which in the presence of water would also liberate iodine from potassium iodide.

Teague (*18*) used iodine pentoxide at 150° C. for the estimation of carbon monoxide in motor-exhaust gas; the method was based on the following reaction:

$$I_2O_5+5CO=5CO_2+2I$$

The iodine from this reaction was collected in aqueous potassium iodide and titrated with 0.001 N sodium thiosulphate. Gane (*8*) studied the oxidation of the volatile emanations from stored apples by passing the air stream over iodine pentoxide heated at 150° C. From the iodine equivalent in terms of carbon dioxide formed during the reaction, Gane concluded that the oxidation was incomplete and that iodine pentoxide could not be used satisfactorily for the estimation of volatile substances produced by apples.

Gane (*8, 10*) finally employed a combustion method to measure the volatile emanation from oranges, apples, and bananas. The method was used in conjunction with respiration studies. After absorption of the respiratory carbon dioxide in flaked sodium hydroxide, the air was passed through a tube of copper oxide heated to 800° C. The organic materials in the air stream were oxidized to carbon dioxide and water; the former was then absorbed in a standardized solution of sodium hydroxide. The rate of air movement through the system did not exceed 0.9 liter per hour, so that the time available for combustion was approximately 1 minute.

SELECTION AND STANDARDIZATION OF OXIDIZING SOLUTION AND SUBSTRATE

Since acetaldehyde has been identified as one of the volatile products liberated from fruit (*13*), and since it can be determined volumetrically with ease and accuracy (*16*), it was used as the reference standard for establishing the optimum conditions for the oxidation of typical volatile emanations from fruit. Ceric sulphate was chosen as the oxidizing agent because of its powerful oxidizing action (*4*), versatility (*15, 21*), stability (*20*), and adaptability to the problem.

The oxidizing solution, 0.1 N in ceric sulphate and 0.5 M in sulphuric acid, was prepared from the anhydrous ceric sulphate and standardized against Bureau of Standards arsenic trioxide, iodine monochloride being used as a catalyst according to the method of Swift and Gregory (*17*). Ceric sulphate solutions of this strength are stable for a year, are not sensitive to light or air, and are not changed by boiling for 1 hour (*20*). Acetaldehyde solutions were standardized according to the bisulphite method of Ripper as given by Sutton (*16*).

Furman and Wallace (*5*) have shown that it is possible to determine thiosulphate in solutions or in salts by titration with standard ceric sulphate solution if an excess of potassium iodide is present. Two precautions must be observed, however: (1) The initial volume for titration should be at least 10 times the volume of thiosulphate used, and (2) the solution for titration must be sufficiently acid (0.3 to 0.5 M in H_2SO_4) to insure a permanent starch iodide end point.

With these precautions in mind, it was possible to obtain comparable standardization values for ceric sulphate by titration with either 0.1 N (Bureau of Standards) arsenic trioxide or with 0.1 N sodium thiosulphate.

The reduction-oxidation changes of ceric sulphate in the experiments reported here were determined by titration against 0.1 N sodium thiosulphate in the presence of excess potassium iodide in acid solution according to the method of Furman and Wallace (*5*).

EXPERIMENTAL PROCEDURE

OXIDATION OF ACETALDEHYDE WITH CERIC SULPHATE

In order to establish the optimum conditions for the oxidation of acetaldehyde, a study was first made of the relative importance of time and temperature. The oxidizing solutions were maintained at a constant volume of 150 cc. and at an acid concentration which was 3 molal in sulphuric acid. The data for these experiments are presented in table 1.

TABLE 1.—*Influence of time and temperature on oxidation of acetaldehyde with ceric sulphate*

CH_3CHO taken (milligrams)	Oxidation		$Ce(SO_4)_2$ (0.1030 N) added	$Ce(SO_4)_2$ (0.1 N) reduced	$Ce(SO_4)_2$ reduced	Reduction-oxidation ratio $\frac{Ce(SO_4)_2}{CH_3CHO}$	Mol. equivalents of $Ce(SO_4)_2$ required per mol CH_3CHO
	Period	Temperature					
	Hours	*° C*	*Cubic centimeters*	*Cubic centimeters*	*Milligrams*		*Milligrams*
14	1	24	25	5.0	166.1	11.86	1.6
14	24	24	25	9.5	315.6	22.53	3.0
14	5	98	50	20.0	664.4	47.45	6.3
14	1	98	50	20.5	681.0	48.64	6.5
14	2	98	50	21.5	714.2	51.00	6.8
14	3	98	50	21.5	714.2	51.00	6.8
14	4	98	50	21.5	714.2	51.00	6.8
0	1	98	50	0			
0	3	98	50	.0			

Oxidation of acetaldehyde occurred at a very low rate at room temperature; less than one-half of the aldehyde had been oxidized after 24 hours at 24° C. Constant titration values, reduction-oxidation ratios, and mol. equivalents of ceric sulphate were obtained, however, after heating at 98° for 2 hours. All subsequent oxidation studies were standardized to a temperature of 98° and for a time interval of 2 hours. Blank titrations showed no reduction of the ceric to the cerous ion under these conditions of oxidation.

In another series of experiments the oxidation of acetaldehyde was studied further by varying the concentration of ceric sulphate and sulphuric acid and the total volume of the oxidizing solution. The temperature and period of oxidation were held at 98° C. and 2 hours, respectively. The results of this study appear in table 2.

The data in table 2 show that the reduction-oxidation ratios (milligrams of $Ce(SO_4)_2$ reduced per milligram of CH_3CHO oxidized) remain constant over a wide range of variation in acid concentration, excess of oxidizing agent, and volume of oxidizing solution. These results are similar to those reported by Willard and Young (*21*) for the oxidation of tartaric acid with ceric sulphate.

Table 2.—*Oxidation of acetaldehyde as influenced by the concentration of $Ce(SO_4)_2$, H_2SO_4, and the total volume of the solution*

Acetaldehyde taken (milligrams)	Total volume	Molarity in H_2SO_4	$Ce(SO_4)_2$ (0.103 N) taken	$Ce(SO_4)_2$ (0.1 N) reduced	$Ce(SO_4)_2$ reduced	Reduction-oxidation ratio $\frac{Ce(SO_4)_2}{CH_3CHO}$
	Cubic centimeters		*Cubic centimeters*	*Cubic centimeters*	*Milligrams*	
14	150	0.5	25.0	20.5	681.0	48.6
14	150	1.0	25.0	21.5	714.2	51.0
14	150	3.0	25.0	21.5	714.2	51.0
14	200	3.0	25.0	21.5	714.2	51.0
14	200	3.0	50.0	21.5	714.2	51.0
14	200	3.0	100.0	21.5	714.2	51.0

A series of oxidations was next carried out in which the acetaldehyde content was varied over a wide range of concentration. The volume of the oxidizing solution was held at 200 cc.; the temperature and reaction time were similar to those for the preceding experiments. Data relative to the oxidation of various concentrations of acetaldehyde appear in table 3.

Table 3.—*Influence of concentration of acetaldehyde on its oxidation with ceric sulphate*

Acetaldehyde taken (milligrams)	$Ce(SO_4)_2$ (0.103 N) taken	$Ce(SO_4)_2$ (0.1 N) reduced	$Ce(SO_4)_2$ reduced	Reduction-oxidation ratio $\frac{Ce(SO_4)_2}{CH_3CHO}$	Acetaldehyde taken (milligrams)	$Ce(SO_4)_2$ (0.103 N) taken	$Ce(SO_4)_2$ (0.1 N) reduced	$Ce(SO_4)_2$ reduced	Reduction-oxidation ratio $\frac{Ce(SO_4)_2}{CH_3CHO}$
	Cubic centimeters	*Cubic centimeters*	*Milligrams*			*Cubic centimeters*	*Cubic centimeters*	*Milligrams*	
44.8	100.0	69.7	2,315.4	51.6	11.2	50.0	17.2	573.4	51.2
22.4	100.0	34.7	1,152.7	51.4	5.6	50.0	8.6	285.7	51.0

Attention should be called to the small change in the reduction-oxidation ratio; it bears a positive correlation with the increase in concentration of acetaldehyde and is probably conditioned by the accuracy of the normality factor of the latter. When due consideration is given to the normality weight of ceric sulphate and to its reduction equivalent in terms of acetaldehyde, the increase in the reduction-oxidation ratio in table 3 becomes less significant. Furthermore, a variation of 700 percent in acetaldehyde concentration produced a concomitant change of only 1 percent in this ratio. Willard and Young (*21*), using a wide range in concentration of tartaric acid, obtained a constant ratio of acid oxidized to ceric sulphate reduced. It is the opinion of the authors that this is also the case with acetaldehyde. The small changes in the reduction-oxidation ratio in table 3 in all probability are representative of the variations that may be anticipated under routine conditions, and the milligrams of ceric sulphate reduced per milligram of acetaldehyde oxidized can be considered constant over a wide range in acetaldehyde concentration.

Since it has been shown (*21*) that formic acid is not further oxidized by ceric sulphate, one would anticipate that acetaldehyde would be oxidized only to formic acid. Theoretically this would require 6 mol-equivalents of cerium according to the following equation:

$$(1)\ 6Ce(SO_4)_2+CH_3CHO+3H_2O \rightarrow 2HCOOH+3Ce(SO_4)_3+3H_2SO_4$$

However, carbon dioxide has been identified as one of the end products in the oxidation of acetaldehyde with ceric sulphate. This fact can be shown in the following equation, where the cerium equivalent is 8:

$$(2)\ 8Ce(SO_4)_2+CH_3CHO+3H_2O \rightarrow HCOOH+CO_2+4Ce(SO_4)_3+4H_2SO_4$$

A cerium equivalent of 6 would require a reduction-oxidation ratio of 45.3, whereas an equivalent of 8 would require a ratio of 60.4. By experiment, however, under standardized conditions, data in tables 1, 2, and 3 show a cerium equivalent of 6.8 and a reduction-oxidation ratio of 51. Obviously stoichiometric relations are not involved in this case and the reaction cannot be expressed by equation. According to Smith [3] what actually happens in this case is that the oxidation of the CHO group is slower than the oxidation of the CH_3 group. Reactions of this type have previously been reported by Willard and Young (*21*).

ABSORPTION AND OXIDATION OF ACETALDEHYDE EMANATIONS

The following experiments are concerned with a quantitative study of the absorption of acetaldehyde in concentrated sulphuric acid and its ultimate determination by oxidation with ceric sulphate. Considerable difficulty was experienced in devising a suitable absorption apparatus to overcome the bothersome problem encountered in the frothing of the sulphuric acid under rapid aeration. Small Truog towers, constructed from heavy glass tubing 22 mm. in diameter and 27 cm. in length, when filled with glass beads and stoppered into 250-cc. extraction flasks, minimized the frothing problem and served as efficient scrubbers.

The amount of acetaldehyde given off was determined by standardizing (*16*) the original solution before and after aeration. The flask containing the acetaldehyde solution was connected to the Truog absorption tower containing 25 cc. of sulphuric acid (specific gravity, 1.84), and the latter was connected through a flowmeter to a vacuum line. The apparatus was held at a temperature of 65° F. and aerated with aldehyde-free air at the rate of 10 liters per hour for a period of 4 days. Four such absorption trains, each bearing a different original concentration of acetaldehyde, were aerated simultaneously.

After aeration for 4 days, the absorption towers were dismantled and the glass beads were washed into the extraction flasks with distilled water. Measured amounts of standardized ceric sulphate were then added and the oxidations were carried out as previously described. Data from these experiments are presented in table 4.

Data in table 4 show that concentrated sulphuric acid is an efficient absorbent for acetaldehyde. Volatiles of this type may be quantitatively removed from an air stream of 10 liters per hour, and may be oxidized directly in the original absorption flask with ceric sulphate. The percentage-recovery values, based on an average ceric sulphate reduction equivalent of 51 mg. for each milligram of acetaldehyde oxidized, are sufficiently great to warrant consideration of this method for the quantitative determination of acetaldehyde. The adaptability of a single acid (H_2SO_4) to the preparation of the ceric sulphate solution, to the absorption and oxidation of the volatile, and even to the final iodometric titration, is a factor of more than passing importance to the analyst.

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[3] Private communication from G. Frederick Smith, University of Illinois, Urbana, Ill.

TABLE 4.—*Aeration of acetaldehyde, absorption in sulphuric acid, and oxidation with ceric sulphate*

CH_3CHO removed by aeration (milligrams)	$Ce(SO_4)_2$ (0.103 N) taken	$Ce(SO_4)_2$ (0.1 N) reduced	$Ce(SO_4)_2$ reduced	Recovery of acetaldehyde based on average R-O ratio of 51	Recovery of acetaldehyde based on that removed by aeration
	Cubic centimeters	*Cubic centimeters*	*Milligrams*	*Milligrams*	*Percent*
6.7	50.0	9.9	328.8	6.43	96.0
12.5	50.0	19.0	631.2	12.37	98.9
25.0	100.0	37.2	1,235.7	24.23	96.9
25.7	100.0	38.4	1,275.6	25.01	97.3
Average recovery					97.2

DETAILS OF PROCEDURE FOR THE PROPOSED METHOD

ABSORPTION OF THE VOLATILES

Small Truog absorption towers, as previously described, filled with glass beads and fitted with 250-cc. extraction flasks, served as efficient gas scrubbers; 25 cc. of concentrated sulphuric acid (specific gravity, 1.84) in these towers has a large absorptive capacity for trapping the volatile emanations from stored fruit. Tests with overripe Bartlett pears have shown the above amount of acid to be sufficient to absorb a quantity of volatiles the oxidation of which would require the reduction of 4.56 gm. of ceric sulphate. The movement of the air stream should be constant and rapid enough to prevent accumulation of the volatiles over the stored fruit.

OXIDATION OF THE VOLATILES

At the close of the absorption period, the towers are dismantled and the glass beads are washed into the extraction flask with 75 cc. of distilled water. Ceric sulphate (0.1 N) is then added; usually 50 to 100 cc. is sufficient for the oxidation and for insuring an excess of the reagent. The resulting solution is approximately 3 molal in sulphuric acid. The flasks are then heated over a boiling water bath for 2 hours (oxidation temperature, 98° C.). At the termination of the oxidation period, their liquid contents are transferred through a glass funnel into a 500-cc. volumetric flask. The glass beads in the funnel are washed with an amount of distilled water sufficient to bring the contents of the volumetric flask to volume. The resulting solution is approximately 1 molal in sulphuric acid.

TITRATION AND EXPRESSION OF RESULTS

Aliquots of 100 cc. from the above-mentioned final volumes are diluted with 250 cc. of distilled water, 0.3 to 0.5 gm. of potassium iodide are added, and the solutions are titrated to a starch iodide end point with 0.1 N sodium thiosulphate. The amount of 0.1 N sodium thiosulphate, by differential titration of blank and sample, is equivalent to the amount of 0.1 N ceric sulphate reduced. This value (in cubic centimeters multiplied by 33.22 milligrams of $Ce\ (SO_4)_2$ per cubic centimeters of 0.1 N solution) will express the results in terms of milligrams of ceric sulphate reduced during the oxidation of the volatile substances. It should again be emphasized that the applicability of this method to the measurement of the total volatile emanation from stored fruit is dependent entirely upon the capacity of such substances for undergoing oxidation.

APPLICATIONS OF THE METHOD

Preliminary studies, involving measurement of the volatile emanation from apples when enclosed in 5-gallon wide-mouthed jars and held at 32° F. in a large commercial storage room, indicated that the source of air during aspiration was an important factor. Attempts to remove the volatiles from storage air prior to passage over the experimental fruit, met with little success. Concentrated sulphuric acid was efficient for only a short period, owing to the high concentration of the volatiles in the storage air and the low solubility of their polymerization derivatives in sulphuric acid at 32°, which solidified after a short interval of aspiration. Outside air, subsequently conditioned as to temperature and humidity, was, however, successfully employed as an aspiration medium.

The volatile materials appearing in an aspiration stream of air from three different sources were determined according to the method presented in this paper. Continuous aerations were maintained for a period of 6 days at the rate of 10 liters of air per hour. These data appear in table 5.

TABLE 5.—*Volatile material in air from different sources*

Source of air	$Ce\ (SO_4)_2$ (0.1 N) reduced	$Ce\ (SO_4)_2$ reduced	Average concentration of volatiles per cubic foot of air (in terms of $Ce\ (SO_4)_2$ reduced)
	Cubic centimeters	*Milligrams*	*Milligrams*
Outside air	0.5	16.61	0.32
Outside air conducted by means of 30 feet of light-walled rubber tubing through a commercial apple-storage chamber filled with fruit held at 32° F.	5.5	182.71	3.59
Inside storage air from same commercial apple-storage chamber	20.5	681.01	13.40

These analyses are in reality blank or check determinations of air under three different conditions. They serve to emphasize the fact that the composition of the aspiration stream may be of major importance, especially in certain studies of the absorption of volatiles from fruit at low temperatures wherein their rate of emanation is greatly restricted.

The data in table 5 are important from three other standpoints: (1) They show the sensitivity of the proposed method; (2) they indicate that rubber is permeable to the odorous constituents given off by stored fruit (rubber is also known to be permeable to ethylene gas);

and (3) they emphasize the degree of accumulation of these materials that may be anticipated within a well-managed commercial apple-storage room.

Respiration measurements have been made during much of the authors' physiological work dealing with the development of soft scald in apples. These studies, up to the present time, indicate no close correlation between the respiratory intensity or its climacteric and this type of storage disorder. Preliminary experiments have shown that it is possible to measure both the carbon dioxide from respiration and the volatile emanation from apples by passing the carbon-dioxide free air of the aspiration stream first through absorption towers containing concentrated sulphuric acid and then through similar ones containing barium hydroxide. By the adoption of this procedure it should be possible to follow the course of the normal ripening of fruits in terms both of respiration and of emanation of volatile materials.

Golden Delicious apples, being both aromatic and susceptible to soft scald, were harvested at their optimum maturity on October 12, 1937, and held at 65° F. Samples of 100 fruits each were removed to 32° at 5-day intervals, and on December 28, 1937, they were examined for soft scald. Determinations of the respiratory activity and the volatile emanations were made on comparable fruit during storage at 65°. These data are shown in figure 1.

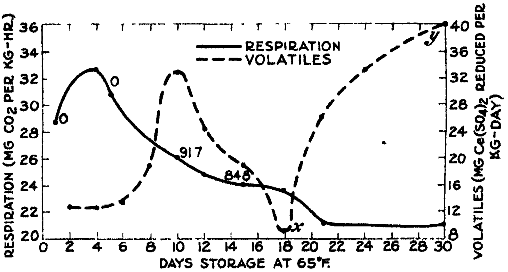

FIGURE 1.—Relation between respiration and emanation of volatiles during ripening of Golden Delicious apples. Numerals on respiration curve indicate percentages of soft scald found December 28, 1937, on fruit that had been removed from 65° F. to 32° after designated number of days. The *x–y* portion of volatile curve represents emanation of volatiles after fruit had become infected with penicillium and botrytis rots.

The rate of respiration of Golden Delicious apples (fig. 1) increased rapidly and reached a climacteric on the fourth day. During this interval, however, there was no apparent change in the rate of liberation of volatile material. Respiration continued to decrease with prolonged ripening and in this respect was typical of fruit in the post-climacteric stage of ripening.

The volatile materials began to increase rapidly after 6 days of storage at 65° F. and reached a climacteric at 10 days. In Golden Delicious apples the respiratory climacteric preceded the period of maximum emanation of volatiles by approximately 1 week. The time of occurrence of these two climacterics assumes an added interest when studied in relation to the subsequent development of soft scald in cold storage. This storage disorder did not occur when Golden Delicious apples were transferred from 65° to 32° during the respiratory climax; maximum amounts of soft scald, however, were subsequently observed in lots transferred from 65° to 32° during the period of their greatest volatile emanation. These relations are receiving further study.

Attention should be called to the x–y portion of the volatile-emanation curve in figure 1. The presence of penicillium and botrytis infection in the experimental fruit was first observed on the eighteenth day of storage. As a precautionary measure to insure the elimination of all fungus spores, the aspiration stream at this time was diverted through a 10-inch glass tube of absorbent cotton prior to absorption in sulphuric acid. The progressive development of these initial infections was strikingly reflected in the subsequent rise in the rate of liberation of the volatile substances. Gane (*9*) has stated that this rapid rise in the emanation of volatile substances with the onset of visual decay is due to injury to the fruit rather than to the byproducts of the micro-organisms. Tissue injury, however, usually produces a temporary stimulation of the respiratory processes, which did not occur in this instance, as shown in figure 1. Whatever may prove to be the cause of the rapid liberation of these volatiles with the onset of fungus attack, it would appear that the proposed method for the determination of these materials may also find application in studies of various factors associated with the growth of certain fungi.

SUMMARY

Acetaldehyde has been oxidized by standard ceric sulphate in sulphuric acid solution, and the excess ceric sulphate has been titrated iodometrically with standard sodium thiosulphate. Optimum conditions for the oxidation have been established. The cerium equivalent of acetaldehyde under these conditions is 6.8, and the reaction is empirical rather than stoichiometric.

Acetaldehyde emanations can be trapped from an air stream of 10 liters per hour by sulphuric acid and subsequently oxidized with ceric sulphate. The average recovery of acetaldehyde under these conditions was 97.2 percent.

Details of procedure are given for a method of measuring the emanation of volatiles from stored fruit. The method is dependent upon the absorption of these materials in concentrated sulphuric acid, oxidation with an excess of standard ceric sulphate, iodometric titration of this excess with sodium thiosulphate, and expression of the results in terms of ceric sulphate reduced.

Certain applications of the method have been presented. They include measurements of the volatile substances in air from various sources, interrelation of respiration and liberation of volatile substances from Golden Delicious apples, and the emanation of volatile materials as influenced by the presence of certain fruit-rot fungi.

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SOIL INFESTATION BY OPHIOBOLUS GRAMINIS AND ITS SPREAD [1]

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INTRODUCTION

The infestation of soil by a soil-borne parasite and the increase and spread of the parasite following introduction constitute a very complex process.

The object of the experiments described in this paper was to determine whether *Ophiobolus graminis* Sacc. may be spread by some of the means commonly known to be effective for similar plant pathogens. These include spread by living plants, by the transportation of infested soil or diseased host particles, by implements, and by the natural action of wind and water.

METHODS

EXPERIMENTAL INFESTATION OF SOIL

The methods used in the experimental infestation of noninfested soils comprised contamination with naturally infested field soil; transfer of infestation by the diseased host, living and dead; and infestation by means of cultures on organic media.

Infested and noninfested soils were brought in contact in several ways in an attempt to simulate possible field infestation such as is produced by soil blowing and by water-borne soil particles. Various measured mixtures of the two soils were also used. Another opportunity for infestation was provided by placing the two soils in contact without mixing.

Tests for the transfer of infestation by the living host plant were made by arranging the experiments in such a way that the organism might progress along living roots from infested to noninfested soil.

The presence of infestation in any soil was indicated by lesions on Kanred wheat grown therein.

Accidental contamination of noninfested soils in the greenhouse was avoided by formaldehyde sterilization of benches and sand beds. Control pots were kept at some distance from infested soil to prevent contamination by water splashing. An experiment was conducted to determine whether accidental contamination was occurring in the greenhouse during the progress of the investigations. Each year the same pots containing noninfested soil were placed among those containing infested soil and it was found that no accidental contamination occurred.

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OBSERVATION AND RATING OF DISEASE SEVERITY

All the disease data on plants infected with take-all are expressed in terms of percentage when possible. These percentage readings were evaluated according to the estimated importance of the particular thing observed in relation to plant injury. For example, death, the maximum injury to a plant, was given a value of 5.0; while the percentage of roots diseased, being much less important, was given a value of 1.0. The various items, with their respective values, are given in the following tabulation. The severity of the lesions on the crown, culms, and roots was rated on a scale of 0 to 100 percent according to the judgment of the observer; the extent of the lesions and the density of their color were the chief determining factors.

Item:	Value
Percentage of plants tillering	0.1
Percentage of culms heading	.1
Percentage of plants dead	5.0
Percentage of crowns diseased	2.0
Percentage of culms diseased	3.0
Percentage of roots diseased	1.0
Percentage of roots retained	.2
Average severity of the disease on crowns	2.5
Average severity of the disease on culms	.1
Average severity of the disease on roots	1.0

The percentage readings were multiplied by the evaluation numbers. The sum of the products that may become greater as the disease becomes more severe (e. g., crowns diseased) was divided by the sum of the products that may become less the more severe the disease becomes (e. g., root retention and tillering). The quotient is the final rating for disease severity. Some observations, such as height of plant, could not be treated in such a manner; therefore the final rating was altered according to the judgment of the worker.

Different environmental conditions may affect such factors as tillering or percentage of culms producing heads, irrespective of the amount of disease present. This source of variation must be taken into account and judged on the basis of the behavior of the controls. In some cases, the omission of certain values is necessary. For example, a few plants in a group may be so badly injured that no culms are formed. In such cases, culm evaluations cannot be used in calculating ratings.

The percentage of culms heading did not appear to be materially affected unless the disease was very severe. Accordingly, this was omitted from the calculations unless the plants were badly injured. Comparison of ratings of similar experiments from year to year was possible only in a general way, as it was not always feasible to make the readings at the same stage of maturity. Furthermore, soils taken from different places in the field and at different times differed in severity of infestation.

A rating of 10 is approximately the dividing line between slight and severe injury.

TRANSFER OF INFESTATION IN SOIL

Contact between infested and noninfested soil is necessary before infestation can be transmitted, and the extent of infestation may be modified by the degree of contact. This can be regulated by mixing various proportions of infested soil with noninfested soil or by arranging the type of contact without mixing. The spread of the infestation through the roots of the host also may be a possibility.

TRANSFER BY MIXING SOILS

When the means by which infested soil may ordinarily be carried to a noninfested portion of a field are considered, it is clear that the resulting mixture will be very dilute. It is thus important to know whether lightly contaminated soil will produce diseased plants, and, if so, to know (1) whether infestation becomes greater with successive cropping, and (2) the readiness and means of fungus spread through the soil.

Tests for the transfer of *Ophiobolus graminis* from one soil to another by mixing were made in two ways, each of which consisted in mixing a given quantity of noninfested soil with infested soil. In the first method, different quantities of infested and noninfested soil were mixed to determine the effect of the proportion of infested soil on subsequent infection. This work was done in the greenhouse. In the second method, the quantity of infested soil in the mixture was very small, being such as might be carried by the natural action of wind and surface water. This work was done both in the field and in the greenhouse.

The ability of lightly contaminated soil to produce badly diseased plants was investigated by mixing infested field soil in various proportions with noninfested soil, usually sterilized, and growing Kanred wheat (*Triticum aestivum* L.; syn. *T. vulgare* Vill.) in it. In one case, the infested soil was mixed with unsterilized noninfested soil for a comparison with sterilized soil. All tests were conducted in the greenhouse.

Infested soil was mixed with noninfested soil in proportions of 75, 66, 50, 33, 25, and 15 percent, by volume. Infested and noninfested soil was used separately for controls. In each test the soil was placed in 8-inch clay pots, and 12 seeds of Kanred wheat, a susceptible, hard red winter variety, were planted in each pot. When the plants in noninfested soil had headed, they were removed and the data on the severity of the disease were recorded. This experiment was continued for 3 years, new lots of infested and noninfested soil being used each year. The data are given in table 1.

TABLE 1.—*Severity of take-all on Kanred wheat plants grown in the greenhouse in noninfested sterilized soil and in various percentages of naturally infested soil mixed with noninfested sterilized soil, Manhattan, Kans., 1931–34*

Type of soil	Tests	Plants	Average height of plants	Plants tillering	Culms		Plants dead	Diseased—			Roots retained	Disease severity			
												Average on—			Final rating
					Total	Heading		Crowns	Culms	Roots		Crowns	Culms	Roots	
	No.	*No.*	*In*	*Pct*	*No*	*Pct.*	*Pct.*	*Pct*	*Pct.*	*Pct.*	*Pct*	*Pct*	*Pct*	*Pct.*	*Pct.*
Noninfested soil	27	324	32.0	59.7	722	56.6	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0
Infested soil (percent):															
100	19	228	17.2	35.4	329	15.0	30.1	94.4	63.5	92.4	32.1	90.0	73.6	84.9	82.4
75	8	91	22.4	22.0	124	22.6	23.9	70.5	56.0	66.6	51.6	71.3	35.3	66.5	50.6
66	8	98	22.9	32.2	130	21.7	20.3	97.7	61.1	92.8	43.0	77.8	50.0	76.2	60.6
50	8	95	25.8	37.8	159	29.6	9.1	73.2	50.8	67.1	64.6	63.9	43.2	64.4	31.2
33	8	94	30.5	26.6	128	66.3	1.0	50.8	22.4	40.3	80.0	41.4	28.8	32.3	14.0
25	8	92	31.0	54.1	195	58.3	1.2	25.6	11.1	18.4	90.4	35.1	20.8	56.1	8.3

The results from pots containing only 15 percent of infested soil are not included in table 1, since only a trace of disease appeared on the roots and there was no appreciable damage to the plants. The results were very similar whether the noninfested soil was sterilized or not. The plants in these experiments were only slightly injured when the proportion of infested soil was less than 33 percent. As moisture conditions in the field are less favorable for the disease than those ordinarily occurring in the greenhouse, undoubtedly a greater proportion of infested soil would be necessary in the field than in the greenhouse for severe damage to take place the first year.

An experiment was conducted in the greenhouse to determine whether the disease severity would increase with repeated cropping of a lightly infested soil. Sterilized soil was mixed with 15 and 30 percent of infested soil. Kanred wheat was grown in the 15-percent mixture in each of 4 years and in the 30-percent mixture in 5 years. The results are given in table 2. In the 30-percent mixture the severity of the disease increased in general but fluctuated considerably from year to year. The wheat was severely injured in the fifth year, but not so much as in the 100-percent infested control soil. There was slight damage in the 15-percent infested soil, but in no case did the final severity rating exceed 3.1.

Experiments to test the possibility of the transfer of the fungus in soil carried by surface water or winds were conducted in the greenhouse and in the field. Water suspensions from infested soil, or dry infested soil, were used as inoculum. Both sterilized and unsterilized noninfested soil was used, the former being sterilized with steam for the greenhouse experiment and with formaldehyde for the field experiment. The water suspensions were prepared by stirring the infested soil vigorously for one-half hour with an abundance of water and pouring off the supernatant, turbid suspension. Two suspensions were used, one being very turbid and the other being allowed to settle for 1 hour. In the greenhouse tests, one application of each of the two suspensions was applied separately to the noninfested soil nearly to the point of saturation and then the soil was mixed and placed in pots in which Kanred wheat was grown.

Two series of these greenhouse experiments were conducted during different years, in which water suspensions of infested soils from different locations were added to unsterilized noninfested soil. In one experiment (series 1a) no disease was produced, while in the other (series 1b) severe disease occurred only where the very turbid suspension had been applied. The detailed data on this experiment are not presented.

In another greenhouse experiment (series 2, table 3) separate soil lots were similarly inoculated with slightly turbid and with very turbid suspensions of infested soil, and two crops of wheat were grown in each of them. In the first crop no disease developed, but in the second it was abundant. The data from the second crop are given in the first part of table 3.

It is evident from these data that under greenhouse conditions infestation carried by water suspensions of infested soil may become well established in 2 crop years. Contrary to the results of the earlier experiment, the noninfested soils to which the slightly turbid suspensions were added became more heavily infested than those inoculated with the very turbid suspensions.

TABLE 2.—*Severity of take-all in five crops of Kanred wheat grown successively in the greenhouse in the same samples of (1) noninfested sterilized soil or (2) noninfested sterilized soil containing different percentages of naturally infested soil, Manhattan, Kans., 1931–36*

Type of soil and crop year	Tests	Plants	Average height of plants	Plants tillering	Culms			Plants dead	Plants having diseased—		Diseased roots	Roots retained	Disease severity			
													Average on—			
					Total	Heading	Average per tillering plant		Crowns	Culms			Crowns	Culms	Roots	Final rating
	Number	*Number*	*Inches*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
Noninfested soil:																
1931–32	6	86	30.6	82.5	249	53.8	3.0	0	0	0	0	100.0	0	0	0	0
1932–33	6	54	30.8	98.1	218	72.0	4.1	0	0	0	0	100.0	0	0	0	0
1933–34	6	49	25.3	53.0	88	79.5	2.5	0	0	0	0	100.0	0	0	0	0
1934–35	6	61	27.8	73.7	146	40.4	2.9	0	0	0	0	100.0	0	0	0	0
1935–36	3	25	27.3	76.0	77	31.3	3.7	0	0	0	0	100.0	0	0	0	0
Total or average	27	275	28.3	76.6	778	55.4	3.2	0	0	0	0	100.0	0	0	0	.0
15 percent of infested soil:																
1932–33	1	10	32.0	100.0	56	53.5	5.6	0	0	0	T[1]	100.0	0	0	5.0	T
1933–34	1	26	23.0	69.2	47	80.8	2.2	0	5.0	0	3.3	98.0	25.0	0	9.0	1.2
1934–35	1	31	23.0	48.3	52	57.6	2.4	0	66.6	0	T	--------	5.0	0	5.0	3.1
1935–36	1	14	23.0	71.4	25	24.0	2.1	0	T	0	T	100.0	T	0	T	T
Total or average	4	81	25.2	72.2	180	53.9	3.0	0	17.9	0	.8	99.3	7.5	0	4.7	[2] 1.8
30 percent of infested soil:																
1931–32	6	29	27.5	41.1	55	40.8	3.2	3.4	59.5	41.6	51.5	64.0	74.3	62.8	65.3	13.8
1932–33	6	45	18.3	82.2	118	18.6	3.0	6.6	100.0	83.0	100.0	31.4	86.1	39.0	87.7	10.8
1933–34	6	36	25.5	0	44	0	--------	0	73.0	0	25.0	95.0	23.0	0	16.3	24.4
1934–35	5	55	27.6	34.5	73	64.3	1.9	12.7	95.3	2.0	25.4	--------	37.5	73.5	56.2	14.6
1935–36	4	35	14.5	11.4	40	(3)	2.3	17.1	100.0	0	78.7	62.5	55.0	0	42.8	49.4
Total or average	27	200	22.6	33.8	330	30.9	2.6	7.9	85.5	25.3	56.1	63.2	55.1	35.0	53.6	[2] 28.4
100 percent of infested soil:																
1931–32	9	70	16.2	31.4	118	16.1	3.2	44.2	100.0	82.4	98.9	23.5	90.0	67.1	83.2	34.9
1932–33	4	33	19.5	21.2	40	12.5	2.0	6.0	100.0	52.4	99.6	45.0	84.0	35.5	78.0	37.0
1933–34	5	56	20.6	5.3	59	89.9	2.0	14.2	89.5	48.8	90.1	55.9	51.0	27.1	61.6	135.6
1934–35	3	33	19.0	15.1	40	12.5	2.4	24.2	100.0	62.6	99.1	--------	79.5	50.5	88.8	60.2
1935–36	6	47	15.0	0	47	17.0	0	14.8	100.0	40.0	100.0	55.8	78.3	35.2	60.4	75.3
Total or average	27	239	18.0	14.6	304	29.6	1.9	20.6	97.9	57.2	97.5	45.1	76.5	43.0	74.4	[2] 61.4

[1] T=trace.

[2] Derived from averages according to tabulation on p. 506.

[3] Too immature for heads.

TABLE 3.—*Severity of take-all on Kanred wheat grown in the greenhouse in nonsterilized or sterilized soil previously inoculated in the field or in the greenhouse, Manhattan, Kans., 1935–36*

Series No.	Soil and soil inoculation	Tests	Plants	Average height of plants	Plants tillering	Culms			Plants dead	Diseased—			Roots retained	Disease severity			
						Total	Heading	Average per tillering plant		Crowns	Culms	Roots		Average on—			Final rating
														Crowns	Culms	Roots	
		Number	*Number*	*Inches*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Pct.*	*Pct.*
2	Noninfested soil sterilized and inoculated in the greenhouse with suspension—18 months previous to observations:																
	Slightly turbid	2	27	19.0	62.9	46	21.7	2.1	7.3	66.6	52.5	65.8	55.2	90.2	66.0	90.2	37.0
	Very turbid	2	17	18.5	10.0	59	38.9	3.5	.0	50.0	.0	49.0	84.0	84.3	.0	38.7	11.8
2	Noninfested soil sterilized and inoculated in the greenhouse with suspension—30 months previous to observations:																
	Slightly turbid	3	24	18.3	95.8	102	50.0	4.4	.0	100.0	62.5	93.3	57.0	73.9	59.3	61.4	46.9
	Very turbid	3	25	18.3	96.0	121	21.4	5.0	.0	80.9	51.8	67.8	61.4	62.5	42.1	57.8	25.2
2	Noninfested soil not sterilized but inoculated in the greenhouse with suspension—30 months previous to observations:																
	Slightly turbid	4	44	15.7	77.2	143	.6	3.9	13.6	100.0	72.9	96.8	53.7	70.3	53.6	64.6	45.0
	Very turbid	2	21	13.0	76.1	71	.0	4.1	.0	100.0	53.8	99.0	66.8	69.2	42.7	49.2	32.8
3	Noninfested soil sterilized and inoculated in the field in 1931 with—																
	Suspension of infested soil	4	45	29.2	53.3	80	48.5	2.5	.0	100.0	.0	16.2	97.5	33.1	.0	15.0	10.6
	Dry infested soil	4	40	30.7	55.0	80	51.2	2.8	.0	91.6	.0	15.0	96.5	34.0	.0	25.0	10.3
3	Noninfested soil not sterilized but inoculated in the field in 1931 with—																
	Suspension of infested soil	4	39	28.2	53.8	77	51.9	2.8	.0	100.0	.0	18.7	94.7	28.7	.0	38.7	11.2
	Dry infested soil	4	40	33.0	47.5	65	61.5	2.3	.0	97.7	.0	15.0	97.2	44.0	.0	31.2	11.6
	Controls grown in—																
	Soil sterilized before seeding in 1935	6	61	27.8	73.7	146	30.8	2.9	.0	.0	.0	.0	100.0	.0	.0	.0	.0
	Naturally infested soil	6	61	12.1	8.1	66	0	2.0	27.8	100.0	75.8	98.8	42.4	77.0	47.2	75.4	103.1

In the field experiments, an area free of infested soil was chosen and four similar plots, each 3 feet square, were spaded and separately enclosed by boards 1 by 8 inches with their edges sunk into the soil about 3 inches. Two of these plots were sterilized with formaldehyde, and the other two were left unsterilized. One of the sterilized and one of the unsterilized plots were inoculated with one application of the very turbid suspension from infested soil applied to the surface with a sprinkling can. The remaining two plots were inoculated with one application of dry, well-pulverized infested soil taken from known infested spots. This pulverized soil was scattered lightly over the surface of the plots and raked into the top ½-inch layer.

The four plots were then cropped with wheat successively for 3 years. During this period no trace of take-all appeared at any time in any of them. Unfortunately, it then became necessary to discontinue the field observations, but the soil was removed to the greenhouse and placed in pots and the cropping with wheat was continued for 2 years. The first crop in the greenhouse (1934–35), the fourth year after inoculation, showed no sign of disease, but the second crop was diseased. The data from this crop (1935–36), together with those for the controls grown in sterilized and naturally infested soil, are given in the last part (series 3) of table 3. The damage was not very great but was sufficient to show that water-borne and wind-blown soil may carry the fungus under field conditions.

TRANSFER BY CONTACT OF SOILS WITHOUT MIXING

Since infested areas in a field often increase in size, it seemed desirable to determine how the fungus finds its way from infested to adjoining noninfested soil and the rate at which this movement takes place. An attempt to study this was made by placing infested and noninfested soils in contact without mixing them. Experiments were conducted both in the field and in the greenhouse.

For the field experiments, two fields known to be free from take-all were selected and four holes 1 foot square and 1 foot deep were dug in each. These holes were filled with infested soil and wheat was planted in this and the surrounding soil. In another noninfested field a hole 12 feet square and 1 foot deep was similarly filled with infested soil, which was cropped to Kanred wheat for 5 successive years.

At the end of the first season, the wheat growing in the 1-foot cubes of infested soil was examined and found to be free of lesions, as was also the wheat in the surrounding soil. In the 12-foot-square plot of infested soil, take-all occurred on the plants the first year in a spot about 4 feet in diameter in the center of the area. The second year a few diseased plants appeared in the center of the same spot. In the 3 following years, all plants were free of take-all.

In the greenhouse experiments, infested soil was arranged in separate boxes so that it was over, under, and against one side of noninfested soil. No wheat was planted, but the soil was kept constantly moist. In one experiment the soils were kept in contact for 2 years and in another for 1 year. In the 2-year experiment the infested soil was renewed at the end of the first year. At the end of the respective periods, the originally noninfested soils were removed in layers or sections taken at increasingly greater distances from the place of contact with infested soil and were tested for the presence of *Ophiobolus graminis* by growing wheat in them. Wheat plants in the noninfested soil never became infected regardless of the position of the noninfested soil with respect to the infested soil or the period of contact.

Wheat was also grown in the infested soil after contact to test its potency for disease production. At the end of the 1-year experiment of eight infested soil samples, four had lost their infestation, one had lost most of it, and three had retained it. In the 2-year experiment the infested soil that had been renewed at the end of the first year retained the infestation.

TRANSFER BY HOST PLANT

It was shown previously[2] that *Ophiobolus graminis* may advance along a wheat root. Accordingly, experiments were planned to find whether the roots may be a means of spreading infestation.

All of the experiments were conducted in the greenhouse. Well-mixed infested soil was placed in boxes (12 by 18 by 4 inches for series 1 to 3 and 12 by 12 by 12 inches for series 4 and 5) in such a way that it was against one side of noninfested soil or over or under it. These arrangements were maintained for 2 successive crop years, but the infested soil was replaced with fresh infested soil between crops. This was done to insure as nearly as possible an ever-present supply of infested soil, since, in greenhouse experiments, as shown above, infested soil in contact with noninfested soil soon lost its infestation.

In series 1, 2, and 3, the 12- by 18- by 4-inch boxes were filled two-thirds of their length with noninfested soil and the remaining one-third with infested soil, care being taken to avoid mechanical mixing at the plane of contact when the boxes were filled For series 4, a 5-inch layer of noninfested soil was placed in the bottom of the 12- by 12- by 12-inch boxes and then a 5-inch layer of infested soil on top of it. In series 5, these layers were placed in the reverse order.

Where infested and noninfested soil were placed in contact side by side, one series (series 1) was left uncropped for 2 years; in a second series (series 2), the infested soil only (series 1a) was cropped for 2 years; and in a third series (series 3), the noninfested soil only (series 3b) was cropped for 2 years. In series 2 and 3 the wheat roots spread to both kinds of soil. In the vertical experiment wheat was planted in one case in infested soil (series 4a) and in the other in noninfested soil (series 5b), with the roots penetrating both soils. The arrangement of the soils and cropping is shown in figure 1. At the end of the period, the soil that was originally noninfested was removed in sections taken at increasingly greater distances from the plane of contact with the infested soil. Each section was potted separately and planted to wheat. Samples of the infested soil used in each arrangement were also potted and cropped with wheat, and the crop was examined in the usual manner for symptoms of take-all.

The results of the experiments in which the infested and noninfested soils were arranged side by side are given in table 4 and those in which they were arranged in layers in table 5. It will be noted in table 4 that, irrespective of the distance from the infested soil, there was no lateral spread of the fungus from infested to noninfested soil unless wheat plants were grown in one or the other with the roots penetrating both soils. This is in agreement with a similar experiment mentioned above. On the other hand, the evidence of spread in the cropped soil to a distance of 12 inches is unmistakable. It is of special interest to note that the fungus spread more when the wheat roots grew from noninfested into infested soil than when they grew from infested into noninfested soil. This was probably due to the greater volume of roots formed by the plants in the noninfested soil, as they were healthy in their early stages of growth while the others were not.

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[2] FELLOWS, HURLEY, and FICKE, C. H. EFFECTS ON WHEAT PLANTS OF OPHIOBOLUS GRAMINIS AT DIFFERENT LEVELS IN THE SOIL. Jour. Agr. Research 49: 871-880, illus. 1934.

The vertical spread from infested to noninfested soil was also much greater when the wheat roots grew from the originally noninfested soil into the infested soil (table 5). In fact, there was practically no vertical spread from infested to noninfested soil when the wheat roots grew from the infested soil into the noninfested soil (series 4b).

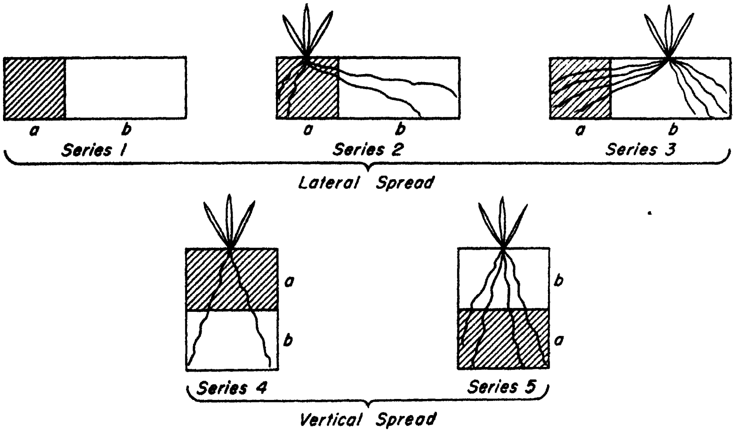

FIGURE 1.—Diagram of methods used in testing the spread of *Ophiobolus graminis* from infested to noninfested soil in infected roots of living wheat plants: *a*, Infested soil; *b*, noninfested soil. (Data in tables 4 and 5.)

The infested soil was not so heavily infested after having been in contact with noninfested soil as it was originally. For example, in the experiment relating to lateral spread (table 4) the original infested soil grew plants with a disease rating of 110.0, whereas after contact with the noninfested soil the disease ratings were 32.6, 44.8, and 67.7 (table 4); in the experiment relating to vertical spread the severity rating after contact was 93.4 and 82.9. These results are in accord with the results of field experiments previously mentioned.

As the plants were grown in soil taken at progressively greater distances from the infested soil against which it rested, a gradual decrease of disease would be expected. In general, this was found to be true but there were some irregularities. These may be due to experimental error or perhaps to the same factors, whatever they are, that sometimes cause infested soils to lose their infestation after having been in contact with noninfested soils.

It is possible that dead material from diseased host plants may harbor the parasite. Such material may be readily carried by farm implements. Large deposits of straw and stubble may accumulate in spots as a result of the periodic cleaning of harrows. The possibility that diseased host remains may carry infestation was tested in

TABLE 4.—*Lateral spread of Ophiobolus graminis in soil as judged by the severity of take-all on Kanred wheat grown in the greenhouse in samples of (1) infested soil and (2) successive, adjoining, lateral sections of originally noninfested soil taken at progressively greater distances from infested soil, Manhattan, Kans.*

Series No.	Location and treatment of soil prior to testing	Tests	Plants	Average height of plants	Plants tillering	Culms			Plants dead	Diseased—			Roots retained	Disease severity			
														Average on—			
						Total	Heading	Average per tillering plant		Crowns	Culms	Roots		Crowns	Culms	Roots	Final rating
		Number	*Number*	*Inches*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Pct.*	*Pct.*
1a	Naturally infested soil not cropped preceding year	4	41	10.5	15.0	53	0	2.3	38.9	100.0	61.1	96.5	60.0	68.9	57.7	62.5	67.7
1b	Sections of originally noninfested noncropped soil adjoining noncropped infested soil:																
	1-3 inches from noncropped infested soil	2	23	17.5	82.9	62	18.1	3.0	0	0	0	0	100.0	0	0	0	0
	4-6 inches from noncropped infested soil	2	23	17.0	75.7	49	20.6	2.5	0	0	0	0	100.0	0	0	0	0
	7-9 inches from noncropped infested soil	2	21	16.5	91.6	57	18.7	2.9	0	0	0	0	100.0	0	0	0	0
	10-12 inches from noncropped infested soil	2	20	17.5	80.0	49	17.2	2.8	0	0	0	0	100.0	0	0	0	0
2a	Naturally infested soil cropped to wheat preceding year	3	25	14.5	17.6	33	0	3.1	18.7	100.0	7.1	53.7	79.0	53.0	5.0	56.2	32.6
2b	Sections of originally noninfested noncropped soil adjoining cropped infested soil:																
	1-3 inches from cropped infested soil	2	23	22.0	24.2	33	59.0	2.4	0	96.1	0	25.0	94.5	13.5	0	37.5	13.5
	4-6 inches from cropped infested soil	2	22	27.5	38.3	34	60.3	2.5	0	50.0	0	2.5	100.0	1.0	0	1.0	4.4
	7-9 inches from cropped infested soil	2	21	24.5	65.9	41	53.2	2.5	0	50.0	0	2.5	100.0	1.0	0	1.0	3.5
	10-12 inches from cropped infested soil	2	21	28.0	61.3	54	43.1	2.8	0	100.0	0	5.0	100.0	2.0	0	3.5	8.1
3a	Naturally infested soil not cropped	3	25	16.0	18.4	33	3.7	2.0	8.3	100.0	41.6	83.7	68.7	61.2	37.0	65.6	44.8
3b	Sections of originally noninfested cropped soil adjoining noncropped infested soil:																
	1-3 inches from noncropped infested soil	2	18	22.0	50.0	35	24.0	3.1	10.0	100.0	67.7	93.9	58.8	65.3	17.0	75.0	47.1
	4-6 inches from noncropped infested soil	2	20	18.0	23.7	27	13.6	2.6	20.1	100.0	99.9	97.5	40.0	80.4	58.6	87.5	96.2
	7-9 inches from noncropped infested soil	2	21	27.5	46.3	35	65.0	2.5	0	100.0	0	57.5	97.5	5.0	0	5.0	11.4
	10-12 inches from noncropped infested soil	2	20	27.9	66.6	45	55.5	3.0	0	6.2	0	0	100.0	5.0	0	0	.9

TABLE 5.—*Vertical spread of Ophiobolus graminis in soil as judged by the severity of take-all in Kanred wheat grown in the greenhouse in samples of (1) infested soil and (2) successive horizontal, 1-inch layers above or below infested soil, Manhattan, Kans.*

Series No.	Location and treatment of soil prior to testing	Tests	Plants	Average height of plants	Plants tillering	Culms			Plants dead	Diseased—			Roots retained	Disease severity			
						Total	Heading	Average per tillering plant		Crowns	Culms	Roots		Average on			Final rating
														Crowns	Culms	Roots	
		Number	*Number*	*Inches*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Pct.*	*Pct.*
4a	Naturally infested cropped soil from above noninfested soil	2	17	19.5	6.2	18.0	38.8	2.0	37.5	100.0	66.6	95.0	47.5	72.2	63.1	75.0	93.4
4b	Layers of originally noninfested soil below infested cropped soil:																
	First 1-inch layer below	2	21	29.5	70.0	34.0	44.0	2.0	0	9.5	0	1.0	100.0	1.0	0	3.0	.9
	Second 1-inch layer below	2	23	31.0	66.2	44.0	38.1	2.8	0	.5	0	.5	100.0	.5	0	.5	.1
	Third 1-inch layer below	2	25	30.0	63.7	62.0	41.8	3.4	0	0	0	0	100.0	0	0	0	0
	Fourth 1-inch layer below	2	20	31.0	80.0	44.0	60.4	2.5	0	0	0	0	100.0	0	0	0	0
	Fifth 1-inch layer below	2	22	29.5	60.0	42.2	59.5	2.5	0	0	0	0	100.0	0	0	0	0
5a	Infested soil below layers of noninfested soil	2	19	17.0	0	19.0	0	0	25.6	100.0	38.5	97.5	46.5	57.8	86.0	77.5	82.9
5b	Layers of originally noninfested, cropped soil above infested soil:																
	First 1-inch layer above	2	22	30.5	77.2	62.0	49.3	3.4	0	50.0	0	10.5	99.0	2.5	0	15.0	4.7
	Second 1-inch layer above	2	24	29.0	84.2	74.0	37.0	3.5	0	78.5	0	22.5	97.5	15.0	0	37.5	9.1
	Third 1-inch layer above	2	20	29.0	87.5	60.0	31.4	3.4	0	62.5	0	3.0	99.5	5.0	0	15.0	5.4
	Fourth 1-inch layer above	2	19	31.0	95.0	71.0	47.6	3.9	0	88.8	0	7.5	99.5	5.0	0	37.5	7.9
	Fifth 1-inch layer above	2	31	27.5	67.6	66.0	45.5	2.7	0	76.6	0	11.0	99.0	5.0	0	37.5	8.0

the field and greenhouse. In an experiment conducted in the greenhouse, stubble and roots from diseased plants grown in the field were mixed with sterilized soil at the rate of 1 part stubble to 3 parts soil by volume. In another experiment, small pieces of leaf sheath carrying the perithecia of *Ophiobolus graminis* were placed on the soil surface at the base of wheat plants in the heading stage. The plants were growing in sterilized soil. In a similar experiment in the field a 2- to 3-inch layer of infested stubble and roots was spaded into the top 6-inch layer of noninfested soil, after which wheat was planted.

In the greenhouse experiments, the plants became badly diseased the first year. All four replications gave the same result. The leaf sheaths with embedded perithecia caused 30.7 percent of the heads to become white. However, there were but few lesions apparent. The same soil later grew two badly diseased wheat crops. In the field experiments, no disease had appeared at the end of 2 years.

INFESTATION OF SOIL BY MEANS OF CULTURES ON ORGANIC MEDIA

Soil may be infested with *Ophiobolus graminis* by introducing into it an organic medium upon which the organism is growing. A sterilized mixture of barley and oat kernels, mixed in the ratio of half and half, has been used successfully. The infested material may be mixed with soil shortly before seeding or it may be placed in the soil adjacent to growing wheat plants at any stage of maturity. Usually, good infection results in the field and greenhouse. Especially heavy infection occurs on seedlings when the soil in which they are grown is infested in this way at seeding time. Within 15 days after emergence, many of the seedlings may be killed. However, when soils thus artificially infested were again seeded to wheat within 2 or 3 months very little infection occurred.

In a single experiment to determine the duration of infestation when accomplished in this manner, 3 parts of sterilized greenhouse soil were mixed with 1 part by volume of the infested medium. Wheat was grown to maturity in the greenhouse for 5 successive years and the crop was badly diseased each year. In a similar experiment in the field the medium was placed in trenches beside growing wheat plants and covered. Diseased plants were produced the first year, but because of unforeseen circumstances the field soil then had to be removed to the greenhouse The first crop, after the soil had been removed to the greenhouse, was not infested. Abundant disease appeared, however, in succeeding years

DISCUSSION AND CONCLUSIONS

From the data presented, it appears possible that take-all may be carried by wind, water, farm implements, spores, the roots of living diseased wheat plants, and by dead diseased material. However, its spread from infested to noninfested soil in the field is a very slow process, fraught with many uncertainties. Even though an infested particle may be carried, it is not assured that infestation will ever increase, if at all, to a sufficient extent to cause disease, for heavily infested soil may lose its infestation in a short time when in contact with noninfested soil. In the greenhouse, where conditions are ideal for the disease, the process is more certain and is greatly speeded up as compared with field conditions. Infestation has disappeared in the greenhouse also when the infested soil was in contact with noninfested soil.

It is rather common, in take-all-infested fields in central Kansas, for the disease to disappear from fields continuously cropped to wheat. For example, the wheat in a certain field was very severely diseased in 1923. This field was cropped to wheat continuously until 1934 without the reappearance of take-all. However, soil removed from this field to the greenhouse in 1930 produced wheat plants with considerable take-all. This also occurred in soil from other fields in which infestation had seemed to disappear.

The ease with which infestation may be secured from spores might appear to be somewhat inconsistent with the slow spread of the disease. This is accounted for by the fact that this fungus does not produce spores prolifically in the Middle West and that the spores when formed are released only under special conditions. Perithecia are seldom found unless the season is exceptionally moist. No doubt spores are an efficient means of spread where they occur, as pointed out by Samuel and Garrett[3] in South Australia. These writers also state that the spores live only 3 or 4 days when dry and that they are ejected under moist conditions only.

The evidence at hand seems to indicate that the most positive and the most frequently operative factor for the establishment, maintenance, and spread of *Ophiobolus graminis*, particularly in the Middle West, is the roots of the living host plant. This is in accord with results recently reported by Padwick,[4] Garrett,[5] and Adam and Colquhoun.[6] In all the greenhouse experiments performed by the writers in which the spread or establishment of infestation occurred, the roots of the host plant were present. In the experiments in which no spread took place, there were no growing wheat roots in the soil. It seems that the spread and establishment of the disease may well proceed in two steps: (1) It is spread as mycelium or spores by any of the mechanical or natural agencies that may be operating; and (2) its establishment in new locations depends largely on the presence of the living host plant.

Further evidence on this point has been presented by Sewell and Melchers,[7] who pointed out that crop rotation is an effective means of controlling take-all. In other words, soil infestation diminishes with the absence of the host plant.

The more or less circular outline of take-all spots in the field indicates uniform spread from a central point. Mycelium, if unobstructed when growing in a medium, forms a circular colony. The roots of a wheat plant are fairly equally distributed through the soil in all directions from the crown.

Consideration of the shape, orientation, and distribution of naturally infested spots in the field may supply information regarding the role of distributing agencies. Thus, if water run-off, farm implements, and wind are factors in distributing infested soil, it would be expected that the relative location and shape of infested areas might reflect this fact. Elongation and frequency of spots appear to have no relation to drainage, direction of prevailing winds, or direction of cultivation. Since the evidence shows that these may be agencies in distribution, the above observations emphasize the fact that it is difficult for the organism to establish itself in new locations.

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[3] SAMUEL, GEOFFREY, and GARRETT, S. D. ASCOSPORE DISCHARGE IN OPHIOBOLUS GRAMINIS, AND ITS PROBABLE RELATION TO THE DEVELOPMENT OF WHITEHEADS IN WHEAT. Phytopathology 23: 721–728. 1933.

[4] PADWICK, G. W. INFLUENCE OF WILD AND CULTIVATED PLANTS ON THE MULTIPLICATION, SURVIVAL AND SPREAD OF CEREAL ROOT-ROTTING FUNGI IN THE SOIL. Canad Jour. Research 12: [575]–589. 1935.

[5] GARRETT, S. D. SOIL CONDITIONS AND THE TAKE-ALL DISEASE OF WHEAT. Ann Appl. Biol. 23. 667–699. 1936.

[6] ADAM, D. B., and COLQUHOUN, T. T. THE SPREAD OF TAKE-ALL THROUGH THE SOIL. Jour. Aust. Inst. Agr. Sci. 2: 172–174. 1936.

[7] SEWELL, M. C., and MELCHERS, L. E. THE EFFECTS OF ROTATION AND TILLAGE ON FOOT-ROT OF WHEAT IN KANSAS, 1920–1924. Jour. Amer. Soc. Agron. 16: 768–771, illus. 1924.

Evidently *Ophiobolus graminis* can live for a period of years in soil cropped to wheat and produce no visible lesions, but if conditions become especially favorable for at least 2 successive years, plants growing in the soil may become infected.

SUMMARY

Experiments were conducted to determine whether *Ophiobolus graminis* may be distributed by the means ordinarily known to carry soil-borne parasites. The data indicate that any agency that can move soil particles or plant debris may carry infestation. However, the establishment of take-all in a new location is slow and uncertain.

In the greenhouse at least 25 percent, by volume, of infested soil in a mixture of infested and noninfested soil was required to produce appreciable disease on the succeeding wheat crop. Successive crops grown in the same mixture became increasingly more severely diseased. Infestation in a mixture containing only 15 percent of infested soil did not increase with 4 years' cropping. The concentration of infestation in either of these mixtures is much greater than would ordinarily result from the transfer of the soil by natural means.

Noninfested soil contaminated with a water suspension from infested soil produced infested wheat plants in the greenhouse when cropped with wheat for 2 years. Infection did not appear during 3 years' cropping in the field; but infection appeared when the soil was removed to the greenhouse and cropped for 2 more years.

Wheat plants, grown in noninfested soil that had been inoculated by applying infested soil lightly to the surface to simulate wind blowing, did not become diseased in the field during 3 years' cropping, but the disease appeared when this soil was brought to the greenhouse and cropped 2 more years.

Ophiobolus graminis did not spread from infested to noninfested soil when such soils were placed in contact without mixing and no wheat roots were growing through the adjacent sections, but when wheat roots were permitted to grow through these sections in either direction, the fungus spread to the noninfested soil. Such spread was greater when the wheat roots grew from the noninfested into the infested soil than when they grew from the infested into the noninfested soil. This was probably due to the greater growth of wheat roots in the noninfested soil.

Infested soils when placed in contact with noninfested soil often lost their infestation both in the field and in the greenhouse. Infestation often apparently disappears from take-all spots in the field.

Dead diseased host remains were a fairly good carrier of infestation in the greenhouse but not in the field.

Spores from the perithecia of *Ophiobolus graminis* were found to be an excellent source of infection, but they are seldom found in the Middle West.

Cultures of *Ophiobolus graminis* grown on a sterilized barley-oat medium were used successfully to infest soil in the field and greenhouse. The soils thus infested remained infested for a long period.

The infected roots of the living host plant are perhaps the most positive factor in carrying *Ophiobolus graminis* to noninfested soil and they contribute greatly to its establishment in a new location. This explains why rotation of crops is an effective control for take-all, particularly as it occurs in the Middle West.

THE FOREST FLOOR OF THE CHAPARRAL IN SAN GABRIEL MOUNTAINS, CALIF.[1]

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INTRODUCTION

The forest floor in the chaparral of southern California as in forests of larger trees has a certain importance in maintaining the productivity of the soil and in such water relations as evaporation, surface run-off, and infiltration. The term "forest floor" is used to designate the total accumulation of organic materials above the mineral soil. The study reported herein was undertaken in an attempt to obtain quantitative measurements of the amount of floor material and its water relations.

REVIEW OF LITERATURE

Earlier determinations of the properties of forest floor from forest stands in the United States and Europe have been summarized by Alway and Zon (*2*).[3] However, none of them originated in the chaparral or its associated prolonged dry summer climate. Maximum dry weights per acre of 119.2 metric tons have been reported for a mixed forest of birch, maple, and spruce in New Hampshire (*8*), and of 87.5 metric tons for spruce, fir, and birch in northern Minnesota (*1*). Low values for well-stocked stands seem to be prevalent in the Southeast where records for mixed hardwoods and shortleaf pine range from 1 to 7 metric tons per acre (*6, 11*) and in Florida, where undisturbed old-growth longleaf pine had only 1.7 metric tons per acre (*3*). In northeastern California, Bodman found 11 metric tons per acre under mature pine and 21 under white fir (*4*). In the mixed conifer forest of the west slope of the Sierra Nevada, 28 metric tons per acre was found by Lowdermilk (*7*). He also recorded 25 tons from the mixed "oak-chaparral" near Berkeley.

Volume weights of 0.26 for pine and from 0.19 to 0.31 for white fir floor were reported by Bodman (*4*).

From the same source, moisture content at cessation of movement ranged from 50 to 85 percent, corresponding to depth of water retained of from 0.15 to 0.3 inch. For the pine-fir floor of the Sierra Nevada, the normal or field moisture capacity averaged 180 percent or 0.26 inch depth of water (*7*). Values for forest-floor material from 100 to as high as 458 percent of the dry weight have been reported (*9*), but it is not certain that the higher figures do not represent moisture content nearer to saturation than to field moisture capacity.

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[1] Received for publication May 2, 1938.

[2] The help of the following assistants at different times in field and laboratory is gratefully acknowledged: W. E. Davis, M. B. Dickerman, H. A. Fowells, P. G. Haddock, W. R. Howden, E. D. Marshall, K. A. Taber, and K. F. Thielking.

[3] Italic numbers in parentheses refer to Literature Cited, p. 534.

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DESCRIPTION OF THE AREAS STUDIED

The field work was done on two areas on the headwaters of tributaries of Big Dalton and San Dimas Canyons in the San Gabriel Mountains of Los Angeles County. More specifically, one area of 240 acres included three small watersheds at the head of Bell Canyon at elevations of from 2,500 to 3,500 feet; and the other, with an area of 128 acres, three watersheds at the head of Fern Canyon at elevations of from 4,500 to 5,400 feet. These areas, originally called the "triplicate" watersheds, are being studied intensively by the California Forest and Range Experiment Station on the San Dimas Experimental Forest. The work here presented has been carried on with the cordial and helpful cooperation of the Station.[4]

In both areas, the topography is steep and mountainous with narrow V-shaped canyons and only minor development of small tributary drainageways. The slopes vary from 15° to 45° and are clothed with a rather shallow mantle of sandy loam to loam soil, in most places, with considerable admixture of gravel and stones.

Except for an occasional narrow strip of canyon bottom, the Bell Canyon watersheds were almost completely denuded of vegetation by the San Gabriel fire of 1919. Consequently, in 1933, when most of the field work was done, the area was occupied by an even-aged stand of chaparral, 14 years old, composed chiefly of chamiso (*Adenostoma fasciculatum*) and *Ceanothus crassifolius*. These species were important components of the chaparral before the fire and they are typical of large areas at similar elevations where the fire histories have varied widely. Fern Canyon had not been burned for more than 55 years, although fire scars on the occasional larger oaks and bigcone spruces testify to earlier fires. The north and east slopes are clothed with a rather dense stand of canyon live oak (*Quercus chrysolepis*) of sprout origin and the south and west slopes with a mixture of manzanita (chiefly *Arctostaphylos glauca*) and *C. divaricatus*.

The sampling points were distributed mechanically over each area with the intention of obtaining a representation from which, by biometric analyses, inferences might be drawn for the area as a whole. Eighty-four sampling points were studied in Bell Canyon and 101 in Fern. At each one the percentage of crown density or coverage was estimated by species on an area of 1 milacre, and the average height and crown or stem diameter of each species was recorded.

A total of 12 species in Bell Canyon and 7 in Fern Canyon made up the principal woody components, various combinations of which formed the chaparral communities or types. These were segregated and characterized by the one or two predominating species and then combined in broader groups if differences between the types were found not to be significant. The first analysis by types, therefore, included 14 communities in Bell and 11 in Fern Canyon.

These differences in the vegetation within either Bell or Fern Canyon might be expected to be reflected in differences in the floor and its water relations. However, differences between Fern and Bell Canyon, which are separated by about 6 miles and some 2,000 feet in elevation, are doubtless associated with differences in soil and climate as well as with differences in vegetation.

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[4] The work was made possible by the allotment of funds from a special State appropriation for research in forestry in cooperation with Federal agencies.

COVERAGE AND DEPTH OF FOREST FLOOR AND CROWN DENSITY

The forest floor, or A_0 horizon, was found to cover from 0.4 to 0.9 of the surface in Bell Canyon and from 0.6 to 1.0 in Fern Canyon, in each case with only three or four exceptions provided by bare spots.

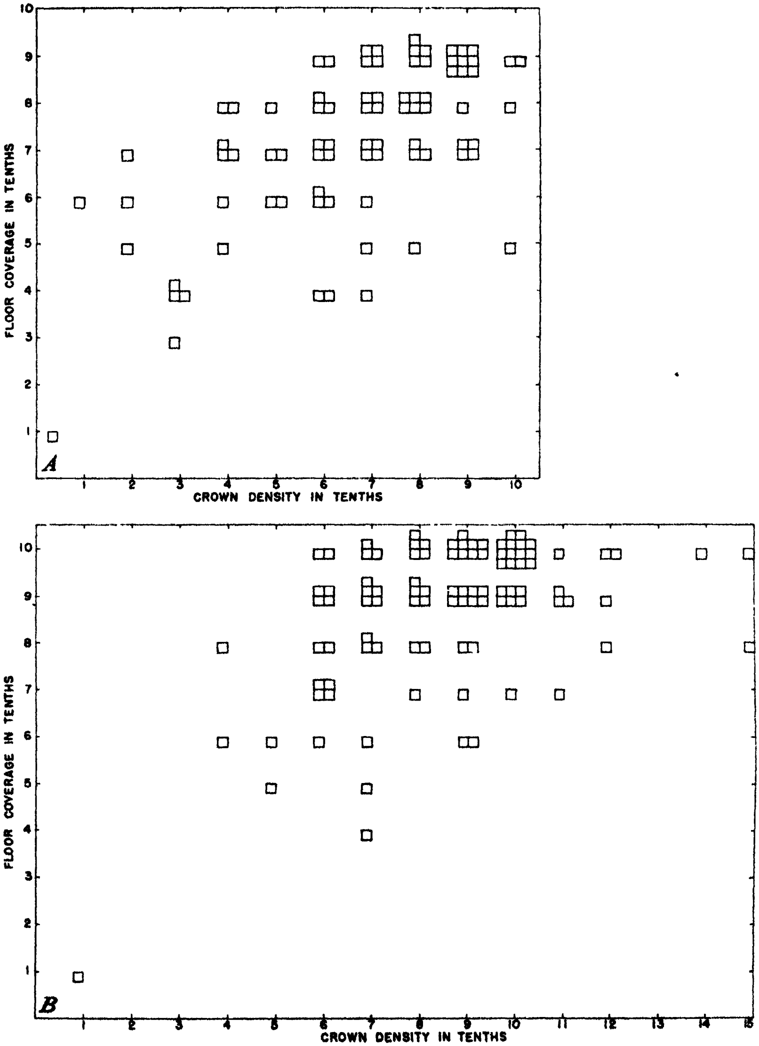

FIGURE 1.—Relation of coverage of forest floor to crown density: *A*, Bell Canyon; *B*, Fern Canyon. Each small square represents one sample.

The distribution of the coverage by the forest floor in relation to the crown density, also expressed on a scale of tenths, is shown in figure 1 for Bell and Fern Canyons. In general the proportion of area covered by the forest floor is somewhat greater than that covered by the crowns. In Bell Canyon, on an average, a floor coverage of 0.8 is associated with a crown density of 0.7, and in Fern Canyon the corresponding figures are 0.9 and 0.8.

In depth the floor varied from 0 to 1 inch, with an average of 0.25 inch in Bell, and up to 2 inches in Fern Canyon, with an average of about 0.6 inch.

AMOUNT OF FOREST FLOOR IN DRY WEIGHT PER ACRE

The amount of the forest floor was determined by collecting samples 1 square foot in area including all the organic accumulation down to the mineral soil, avoiding as far as possible the mineral matter. However, on the steep slopes where there is considerable creep, the separation is difficult to make and samples inevitably include mineral fragments. These were separated by sieving and floating the material in the laboratory; the remaining organic matter was oven-dried for 132 hours at 70° to 80° C.[5] The resulting weights converted to an area of 1 acre will be termed "dry weight per acre."

These dry weights of single samples varied from 0.0 to 20.4 metric tons per acre for Bell Canyon, and up to 37.6 for Fern Canyon. The averages by chaparral types ranging from 2.9 to 21.2 metric tons per acre are shown in tables 1 and 2. The figures for number of samples show the distribution of sampling points by types and, since the sampling points were mechanically spaced, they give an indication of the relative abundance of the different types. Thus the types that occupy only small areas are represented by only a few samples and the averages for these less extensive types are correspondingly less reliable.

TABLE 1.—*Dry weight of forest floor in Bell Canyon*

<table>
<tr><th rowspan="2">Group and chaparral type [1]</th><th rowspan="2">Means of dry weight per acre</th><th rowspan="2">Standard error</th><th rowspan="2">Coefficient of variation</th><th rowspan="2">Samples</th><th colspan="5">Significance of differences between means [2]</th></tr>
<tr><th>Sm-Eff</th><th>Eff</th><th>Qd-Ccr</th><th>Af-Ccr</th><th>Af-Sm</th></tr>
<tr><td></td><td><i>Metric tons</i></td><td><i>Metric tons</i></td><td><i>Percent</i></td><td><i>Number</i></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>High group:</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Ro [1]</td><td>12.33</td><td>4.02</td><td>45.9</td><td>2</td><td>s</td><td>s</td><td>n</td><td>h s</td><td>n</td></tr>
<tr><td>Gv</td><td>9.50</td><td></td><td></td><td>1</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Sm-Ccr</td><td>7.40</td><td>1.72</td><td>52.0</td><td>5</td><td>n</td><td>n</td><td>n</td><td>s</td><td>n</td></tr>
<tr><td>Pa</td><td>7.22</td><td>1.29</td><td>30.8</td><td>3</td><td>s</td><td>n</td><td>n</td><td>n</td><td>n</td></tr>
<tr><td>Ccr-Sm</td><td>6.72</td><td>1.71</td><td>84.3</td><td>11</td><td>n</td><td>n</td><td>n</td><td>n</td><td>n</td></tr>
<tr><td>Cb</td><td>6.23</td><td>3.92</td><td>88.8</td><td>2</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Co-Qwf</td><td>5.74</td><td>2.92</td><td>71.8</td><td>2</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Medium group:</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Ccr-Af</td><td>5.17</td><td>1.02</td><td>89.5</td><td>21</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Af-Sm</td><td>4.82</td><td>1.47</td><td>74.3</td><td>6</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Af-Ccr</td><td>4.21</td><td>.58</td><td>55.4</td><td>16</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Ag</td><td>4.13</td><td></td><td></td><td>1</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Qd-Ccr</td><td>3.75</td><td>1.14</td><td>60.2</td><td>4</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Low group:</td><td></td><td></td><td></td><td></td><td colspan="2" rowspan="3">Medium and low</td><td rowspan="3">Low</td><td colspan="2" rowspan="3">Medium</td></tr>
<tr><td>Eff</td><td>3.27</td><td>.80</td><td>54.1</td><td>5</td></tr>
<tr><td>Sm-Eff</td><td>2.92</td><td>.85</td><td>65.0</td><td>5</td></tr>
<tr><td>Group means:</td><td></td><td></td><td></td><td></td><td colspan="2"></td><td></td><td colspan="2"></td></tr>
<tr><td>High</td><td>7.33</td><td>1.00</td><td>69.2</td><td>26</td><td colspan="2">h s</td><td>s</td><td colspan="2">s</td></tr>
<tr><td>Medium</td><td>4.67</td><td>.53</td><td>78.5</td><td>48</td><td colspan="2">n</td><td>n</td><td colspan="2">n</td></tr>
<tr><td>Low</td><td>3.10</td><td>.59</td><td>59.6</td><td>10</td><td colspan="2"></td><td></td><td colspan="2"></td></tr>
<tr><td>Medium and low</td><td>4.40</td><td>1.01</td><td>79.1</td><td>58</td><td colspan="2"></td><td></td><td colspan="2"></td></tr>
<tr><td>All types</td><td>5.31</td><td>.46</td><td>79.3</td><td>84</td><td colspan="2"></td><td></td><td colspan="2"></td></tr>
</table>

[1] Abbreviations as here used are as follows: Af, *Adenostoma fasciculatum*, chamiso; Ag, *Arctostaphylos glauca*, manzanita; Cb, *Cercocarpus betuloides*, mountain-mahogany; Ccr, *Ceanothus crassifolius*; Co, *C. oliganthus*, hairy ceanothus; Eff, *Eriogonum fasciculatum* var. *foliolosum*, wild buckwheat; Gv, *Garrya veatchii*, silktassel; Pa *Photinia arbutifolia*, christmasberry; Qd, *Quercus dumosa*, scrub oak; Qwf, *Q. wislizenii* var. *frutescens*, interior live oak; Ro, *Rhus ovata*, sugar sumac; Sm, *Salvia mellifera*, black sage.

[2] s=significant; hs=highly significant; n=not significant.

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[5] The dry weights thus obtained were found by test to be from 0.3 to 0.9 percent higher than those in an oven at 105° C. The lower temperature was used to avoid losses by combustion.

TABLE 2.—*Dry weight of forest floor in Fern Canyon*

Group and chaparral type [1]	Means of dry weight per acre	Standard error	Coefficient of variation	Samples	Significance of differences between means [2]				
					Pm	Qc	Qwf	Qc-Qwf	Qc-Ag
	Metric tons	*Metric tons*	*Percent*	*Number*					
High group:									
Ag [1]	21.18	3.70	49.4	8	n	h s	n	n	
Ag-Af	20.56	3.89	42.3	5	n	s	n	n	
Ag-Cd	20.16	1.84	32.8	13	s	h s	s	s	n
Cd-Ag	19.74	2.93	33.2	5	s	n	n	n	
Medium group									
Qwf-Ag	17.57	4.02	51.1	5	......	n			
Qc-Ag	15.42	3.53	56.1	6					
Low group:									
Qc-Qwf	12.84	2.21	45.6	7					
Qwf	12.29	1.72	36.9	7					
Qc	12.24	3.76	67.6	40	Low	Medium			
Pm	10.11	1.50	25.3	4					
Af	4.69	....	....	1					
Group means:									
High	20.42	1.46	39.8	31	h s	n			
High and medium	19.37	1.31	43.9	42	h s				
Medium	16.40	2.67	54.1	11	n				
Low	12.04	.90	58.2	59					
All types	15.09	.84	56.4	101					

[1] Abbreviations used: Ag, *Arctostaphylos glauca*, manzanita; Af, *Adenostoma fasciculatum*, chamiso; Qc, *Quercus chrysolepis*, canyon live oak; Cd, *Ceanothus divaricatus*; Qwf, *Quercus wislizenii* var. *frutescens* interior live oak; Pm, *Pseudotsuga macrocarpa*, bigcone-spruce.

[2] See footnote 2, table 1.

The point that stands out in these figures is that the differences in the average weight of floor in different types are small. These differences were tested for significance by the *F* test in the analysis of variance as outlined by Snedecor (*12*), and the expectation was verified that many of the types are not significantly different one from another in average dry weight of floor per acre. The significance of the difference is indicated in the right-hand columns of the tables, where the types or groups from the low end of the series are repeated as headings of the columns to form a cross classification with the types or groups of the high end of the series in the left-hand column. On that basis the types were combined into three groups for each of the two canyons in such a way that differences between groups would be significant and, at the same time, the groups would include types that seemed to have some logical interrelation.

The 12 species that are commonly represented in Bell Canyon and give character to the chaparral communities occur in various mixtures in which the relations to site or to natural succession are not well defined. Almost all the species were included by Cooper (*5*) as components of the chaparral climax. If they have successional relations, the 14 years since the last fire has not been sufficient for their expression. It seems likely that the *Rhus* and *Garrya* and *Photinia*, which have heavy accumulations of forest floor, may represent more persistent species in succession than the *Eriogonum* and chamiso (Af), which have light forest floors. Actually, by the test of significance, the *Rhus* with an average of 12.33 metric tons per acre is significantly different from the *Eriogonum* types with less than 3.3 metric tons, and from the chamiso—*Ceanothus crassifolius* type with 4.21 metric tons. This latter type is also significantly different from the type in which the black sage (Sm) is dominant in association with the *Ceanothus* and in which the forest floor averages 7.4 metric tons. The only other case of a significant difference between means is that of Christmasberry (Pa) with 7.22 metric tons, as compared with the black sage and *Eriogonum* with 2.92 metric tons. Obviously there is little justification for distinguishing the 14 types on the basis of the amount of forest floor.

When they are combined in three groups, some of the homogeneity of the individual types is lost, with a compensating gain in differentiation. The low group, with an average dry weight of forest floor of 3.1 metric tons, is characterized by the *Eriogonum;* the intermediate group, with 4.67 metric tons, contains the types with chamiso; and the high group, with 7.33 metric tons, a variety of types including those which have been suggested as more persistent. Between the means of these three groups, the high is significantly different from both the medium and the low, but the intermediate group is not significantly different from the low. Going a step further, therefore, and combining the intermediate and low groups, with further loss of homogeneity, there remains a high group with a mean dry weight of 7.33, which is significantly different with a probability of 100 to 1 from the combined low group with a mean of 4.4 metric tons per acre.

The variation within the types and in the groups is high throughout, as indicated by the magnitudes of the coefficients of variation, which range from 30.8 to 89.5 percent. The means of the two groups, high and medium and low, have standard errors of 1 metric ton per acre, which may be interpreted by saying that another sample of the same size from watersheds with similar vegetation would give means for the high group ranging between 6.3 and 8.3 and for the low group between 3.4 and 5.4 metric tons, with a probability of 68 in 100.

In Fern Canyon at elevations between 4,500 and 5,400 feet, the means for the forest floor in the different types range from 4.7 metric tons for chamiso (Af) to 21.18 for manzanita (Ag). The four types in which manzanita occurs pure or in mixture with chamiso or *Ceanothus divaricatus* are higher, and usually significantly higher, in dry weight of floor than the types characterized by oak or spruce or chamiso. If three groups of types are distinguished, only the high group of manzanita types is distinct from the low group with the oak (Qc, Qwf), bigcone-spruce (Pm), and chamiso. If the two types in which manzanita and oak are associated and the other types in which manzanita is present are combined, the resulting group with a mean weight of forest floor of 19.37 metric tons differs with high significance from the low group with 12.04 metric tons.

Again the variation within types and groups is high, ranging from about 25 to 68 percent and the standard errors of the group means, when only two groups are distinguished, are 1.3 metric tons for the high and medium and 0.9 for the low group. In this case, however, these two groups, the high one characterized by manzanita and the low one by oak or spruce, are quite definitely associated with a difference in site. The former is found on the south and west aspects, while the latter occurs on the north and east aspects and in the canyon bottoms.

When the 14-year-old chaparral of Bell Canyon below 4,000 feet is compared with the more than 50-year-old vegetation of Fern Canyon above 4,000 feet, the difference between 5.3 and 15.0 metric tons per acre dry weight of forest floor is highly significant.

An attempt was made to investigate the relation of the amount of forest floor to the foliage volume. As an expression of foliage volume, the product of the average height of the vegetation in feet and the crown density in tenths was used. This gave a scale ranging from less than 1 to 35. In Bell Canyon, where the foliage or crown length closely approached the height of the chaparral, there was a poorly defined tendency for the dry weight of forest floor to increase in a somewhat linear trend as the foliage volume increased. For separate groups of the chaparral types, however, the trend disappeared. In Fern Canyon, nothing that could be called a trend was found, perhaps because, with the oaks particularly, the total height was not a good index of crown length. In any case, no evidence was found of a usable relation between dry weight of forest floor and foliage volume.

VOLUME WEIGHT OF FOREST FLOOR

The volume weight of the forest floor, determined as the ratio of the oven-dry weight to the weight of an equal volume of water, provides a measure of the density of the floor material and might be expected to reflect differences in the moisture relations or in the composition of the chaparral as it influences the underlying mineral soil. The term "volume weight" is used as a synonym for "apparent specific gravity." The volume weights were obtained from the dry weights and the measurement of average depth of the square-foot samples from which volumes were computed. The average volume weights of the different chaparral types in Bell and Fern Canyons, together with the biometric analysis, are shown in tables 3 and 4.

Table 3.—*Volume weights of forest floor in Bell Canyon* [1]

Group and chaparral type	Volume weight	Standard error	Coefficient of variation	Samples	Significance of differences between means				
					Cb	Qd-Cer	Co-Qwf	Sm-Eff	Cer-Sm
High group			*Percent*	*Number*					
Pa	0.27	0 02	13.6	3	s	n	s	n	n
Af-Sm	.26	.05	48 4	6	n	n	n	n	
Ro	.26	004	2 8	2	s	n	s	n	
Sm-Cer	.25	.03	25.6	5	s	n	n		
Af-Cer	.24	.03	43 7	15	n	n			
Medium group									
Eff	.23	.02	13.9	4	s	n	n		
Cer-Af	.22	.01	50.9	21					
Cer-Sm	20	.03	58 3	11					
Sm-Eff	.18	.05	61.6	5					
Low group									
Co-Qwf	.16	.02	22 6	2					
Qd-Cer	.15	.05	69.5	4					
Gv	.09	----------	----------	1					
Cb	.08	.02	38.1	2	Low	Medium			
Ag	.08	----------	----------	1					
Group means:									
High	.25	.02	38 9	31	h s	n			
Medium	.21	.02	51.4	41	s				
Low	.13	.02	61.1	10					
All types	.21	.01	49 5	82					

[1] For meaning of abbreviations see footnotes, table 1.

In Bell Canyon the range is from 0.08 to 0.27. The differences in the means between different types are small and consequently only a few of the higher ones are significantly different from the lower ones. Such significant differences as are found are between the less extensive types, such as Christmasberry (*Pa*) or *Rhus*, with volume weights of

TABLE 4.—*Volume weights of forest floor in Fern Canyon* [1]

Group and chaparral type	Volume weight	Standard error	Coefficient of variation	Samples	Significance of differences between means					
					Qc-Ag	Qwf-Ag	Qc	Qwf	Qc-Qwf	Ag
High group:			*Percent*	*Number*						
Af	0 36	---	---	1	---	---	---	---	---	---
Ag-Cd	29	0.02	20 4	13	h s	h s	h s	h s	s	n
Ag-Af	.28	01	7 2	5	h s	h s	s	n		
Cd-Ag	.27	.03	27.4	5	s	n	n			
Pm	.25	.02	17 9	4	s	n	n			
Ag	.25	02	25 2	8	s	n				
Low group:										
Qc-Qwf	.21	.03	42.0	7						
Qwf	.20	.03	35 5	7						
Qc	.19	.01	42 7	39						
Qwf-Ag	.18	02	27 7	5						
Qc-Ag	.17	.02	28 4	6						
Group means					Low					
High	.27	01	22 6	36	h s					
Low	.19	.01	40 4	64						
All types	.22	.01	36.9	100						

[1] For meaning of abbreviations see footnotes, tables 1 and 2.

0.27 and 0.26, and *Cercocarpus betuloides* (Cb) or *Ceanothus oliganthus* and oak (Co–Qwf), with volume weights of 0.08 and 0.16.

The sequence of the types in descending order of the mean volume weights is not the same as was found in the analyses of dry weight or of percentage field moisture capacity or of depth of water retained. Correspondingly, the groups that can be formed on the basis of volume weight are not the same as those distinguished in the other analyses. However, if the groups on any other basis were used, there would be neither homogeneity within nor significant differences between them. It seems better, therefore, to differentiate the groups independently for volume weight; and for that purpose three groups designated "high," "medium," and "low" are distinguished. The high group, with mean volume weight of 0.25, coefficient of variation of 38.9 percent, and standard error of 0.02, contains the types in which chamiso (Af) predominates, together with Christmasberry, *Rhus*, and the black sage (Sm) with admixture of *Ceanothus crassifolius*. The intermediate group includes the two types in which *C. crassifolius* is dominant and the two in which *Eriogonum* is found. This group has a mean volume weight of 0.21, coefficient of variation of 51 percent, and standard error of 0.02. The low group includes the two types in which oak (Qd, Qwf) is an element, together with the *Garrya*, manzanita (Ag), and *Cercocarpus betuloides*.

As between these groups, the low is different from the high group with high significance, and is also significantly different from the medium group. The high and medium groups are not significantly different one from another. The coefficients of variation expressing the dispersion of the individual samples making up a type or group, and the standard errors representing the value of the means for purposes of generalization or prediction are not very different for the groups from their values for the types. In other words, the subdivision of the vegetation into a larger number of types does not increase either the homogeneity of the units or the value of the means for application to larger areas of similar vegetation.

In Fern Canyon, the volume weights for the chaparral types vary from 0.17 to 0.36. In general the variation within types is less than was the case in Bell Canyon, as might be anticipated from the fact that the types in Fern Canyon have had an opportunity to develop characteristic properties undisturbed over a much longer period of time. Although the range of the mean volume weights and the differences between individual types are not greater than in Bell Canyon, the types with high volume weight are in many cases significantly different from those with low. Thus the types with manzanita (Ag) are significantly different, and in several cases with probabilities of more than 99 in 100, from the types in which either oak (Qc, Qwf) predominates.

These types on the bases of biometric analysis, composition, and site fall rather definitely into two groups. The high group contains the mixtures of manzanita, chamiso (Af), and *Ceanothus divaricatus*, together with the bigcone-spruce (Pm). This group has a mean volume weight of 0.27 with a coefficient of variation of 22.6 percent and standard error of 0.01. The low group contains all of the oak types and their mixtures and has a mean volume weight of 0.19 with a coefficient of variation of 40.4 percent and a standard error of 0.01. The satisfactory basis for the separation of these two groups is confirmed by the finding that the difference between their means is highly significant.

The mean volume weight of 0.21 for all of the samples from Bell Canyon is not significantly different from the closely corresponding value of 0.22 for Fern Canyon.

FIELD MOISTURE CAPACITY

The field moisture capacity of the floor samples was determined in the laboratory by a modification of the method proposed by Shaw (*10*). After the mineral matter had been separated and removed, the sample was placed in a metal cylinder and immersed in water for 48 hours. The cylinders were then removed and allowed to drain for a few minutes until the freely flowing water had drained off. They were then placed on boxes of dry loam soil, the floor material being separated from the mineral soil of the boxes by a layer of cheesecloth. The tops of the cylinders were sealed with waxed cloths to prevent loss of moisture by evaporation. The samples were left to drain in this way for 48 hours, when the floor was removed and all or a portion of it dried as a basis for expressing the field moisture capacity as a percentage of the oven-dry weight.

This method was found to give results not reproducible within about 10 percent. Tests were made of the effect of resoaking and running samples a second time, of using a sandy loam in comparison with the loam soil, of setting only one instead of three cylinders in a single soil box, and of rearranging the forest-floor material in the cylinders. None of these tests showed any consistent effect on the amount of moisture retained, or on the percentage of field moisture capacity. It seems reasonable to conclude, therefore, that, although individual determinations are not closely reproducible, the means of several determinations within a type or group are reliable as indicating the retention of capillary moisture by the floor.

As in the case of dry weights, the field moisture capacity was first investigated for the individual chaparral types. The figures are shown in tables 5 and 6.

TABLE 5.—*Field moisture capacity of forest floor in Bell Canyon* [1]

Group and chaparral type	Mean field moisture capacity	Standard error	Coefficient of variation	Samples	Significance of differences between means			
					Ccr-Af	Af-Ccr	Ccr-Sm	Af-Sm
	Percent	*Percent*	*Percent*	*Number*				
High group:								
Co-Qwf	191	18	14	2	h s	h s	n	n
Eff	190	18	19	4	h s	h s	n	n
Ro	178	5	4	2	s	s	n	n
Medium group:								
Qd-Ccr	165	21	25	4	n	n	n	n
Pa	164	11	11	3				
Cb	162	26	22	2				
Sm-Ccr	161	7	10	5				
Sm-Eff	159	8	11	5				
Af-Sm	147	10	16	6				
Ccr-Sm	145	11	26	11				
Low group:								
Gv	140			1				
Af-Ccr	139	5	13	15				
Ccr-Af	139	4	14	21				
Ag	125			1				
					Low	Medium		
Group means:								
High	187	10	16	8	h s	s		
Medium	154	5	20	36	s			
Low	139	3	13	38				
All types	150	3	20	82				

[1] For meaning of abbreviations see footnotes, table 1.

TABLE 6.—*Field moisture capacity of forest floor in Fern Canyon* [1]

Group and chaparral type	Mean field moisture capacity	Standard error	Coefficient of variation	Samples	Significance of differences between means					
					Ag-Cd	Ag-Af	Cd-Ag	Ag	Qwf-Ag	Qc-Ag
	Percent	*Percent*	*Percent*	*Number*						
High group:										
Qc	173	4	15	40	h s	h s	s	s	s	n
Qc-Qwf	171	10	15	7	h s	s	s	n	n	
Pm	167	16	19	4	s	n	n	n		
Medium group:										
Qwf	158	12	19	7	s	n	n	n		
Qc-Ag	154	15	25	6	n	n	n	n		
Qwf-Ag	148	8	12	5						
Low group:										
Ag	144	9	18	8						
Cd-Ag	138	6	10	5						
Af	138			1						
Ag-Af	135	8	14	5						
Ag-Cd	130	5	15	13						
					Low	Medium				
Group means:										
High	172	4	16	51	h s	s				
Medium	154	7	20	18	s					
Low	136	4	15	32						
All types	157	3	19	101						

[1] For meaning of abbreviations see footnotes, tables 1 and 2.

In general, the two predominating types in Bell Canyon, composed of mixtures of chamiso (Af) and *Ceanothus crassifolius*, and the four types with strong admixtures of black sage (Sm), tend to have a low field moisture capacity, that is, about 140 percent of the dry weight; whereas the oak (Qd, Qwf), *Eriogonum*, *Rhus*, and Christmasberry (Pa) have high average percentages ranging from 164 to 191. The total range for the types in Bell Canyon is only from 125 to 191 percent. The standard errors of the means range from 4 to 26 percent. The coefficients of variation for the individual types, ranging from 4 to 26 percent, are fairly low. This is in contrast to the high variability which was found for the dry weights and other properties. Considering the limited range and the small differences between individual types, it is not surprising that most of them are not significantly different, one from another. Only the types with chamiso and *C. crassifolius* at the low end are significantly different from the *C. oliganthus*-oak, *Eriogonum*, and *Rhus* types at the high end.

When the three last-named types are combined in a group with an average field moisture capacity of 187 percent, this group is significantly different from the low group containing the chamiso-*Ceanothus crassifolius*, and single samples of the manzanita (Ag) and *Garrya* types, and also from the intermediate group containing the seven other types. These groups have coefficients of variation from 13 to 20 percent and standard errors from 3 to 10 percent.

Between the field moisture capacities and the amounts of forest floor, there is no definite relation either in the individual types or in the composition of the groups.

In Fern Canyon, the range of the means of field moisture capacity for individual types is from 130 to 173 percent. The five types at the lower end are those which have no oak and in which manzanita (Ag) is an important element, usually in mixture with chamiso (Af) or *Ceanothus divaricatus*. Canyon live oak (Qc) heads the list with 173 percent, and the mixture of canyon and interior live oaks (Qwf) and bigcone-spruce (Pm) are the next two in order. Notwithstanding the small range and small differences between mean field moisture capacities of the different types, the canyon live oak and spruce types are significantly different from most of the manzanita types. The coefficients of variation for individual types are again low, ranging from 10 to 25 percent and the standard errors of the means from 4 to 16 percent.

The types may be combined in three groups, a high group with canyon live oak or bigcone-spruce and without manzanita, with an average field moisture capacity of 172 percent; a low group, including four manzanita types without oak, having a field moisture capacity of 136 percent; and an intermediate group in which interior live oak or the mixture of oak and manzanita are represented, with a mean field moisture capacity of 154 percent. These three groups are significantly different, one from another. The coefficients of variation are from 15 to 20 percent; and the standard errors, from 4 to 7 percent.

In this instance of Fern Canyon, it is evident that the field moisture capacities of the different types tend to be inversely proportional to the dry weights of the forest floor; that is, the manzanita types which have high weight of floor have low field moisture capacities; and the oak and bigcone-spruce types which have low weights of forest floor tend to have high field moisture capacities.

When the forest floors of Fern and Bell Canyons are compared, the field moisture capacities are not significantly different.

DEPTH OF WATER RETAINED

If the weights of the forest-floor samples are multiplied by the percentages of water retained at field moisture capacity, and the product divided by a suitable constant, figures are obtained for the inches depth of water retained by the forest floor at field moisture capacity on the basis of oven-dry weight. The results of these computations, together with the biometric analysis, are shown for the different chaparral types and groups for Bell Canyon in table 7 and for Fern Canyon in table 8.

TABLE 7.—*Depth of water retained by forest floor at field moisture capacity in Bell Canyon*[1]

Group and chaparral type	Water at field moisture capacity	Standard error	Coefficient of variation	Samples	Significance of differences between means				
					Sm-Eff	Qd-Ccr	Af-Ccr	Eff	Ccr-Af
High group.	*Inches*	*Inches*	*Percent*	*Number*					
Ro	0 21	0.06	43	2	n	n	s	n	n
Gv	.13	------	------	1	------	------	------	------	------
Sm-Ccr	12	03	61	5	n	n	s	n	n
Cb	.12	08	93	2	n	n	n	n	
Co-Qwf	.12	.07	78	2	n	n	n	n	
Pa	.11	02	28	3	s	n	s	n	
Ccr-Sm	.10	.02	79	11	------	------	n		
Low group.									
Af-Sm	.07	.03	82	6					
Ccr-Af	07	02	93	21					
Eff	06	.02	60	5					
Af-Ccr	06	.01	60	15					
Qd-Ccr	.06	.02	57	4					
Ag	05	------	------	1	Low				
Sm-Eff	.04	.01	63	5					
Group means.									
High	12	02	75	26	hs.				
Low	06	.01	83	57					
All types	.08	.01	87	83					

[1] For meaning of abbreviations see footnotes to table 1.

TABLE 8.—*Depth of water retained by forest floor at field moisture capacity in Fern Canyon*[1]

Group and chaparral type	Water at field moisture capacity	Standard error	Coefficient of variation	Samples	Significance of differences between means		
					Pm	Qwf	Qc
High group:	*Inches*	*Inches*	*Percent*	*Number*			
Ag	0.29	0 04	43	8	n	n	n
Cd-Ag	.26	.04	33	5	n	n	
Ag-Af	.26	.05	40	5	n	n	
Qwf-Ag	.26	.07	62	5	n		
Qc-Ag	.26	.08	74	6			
Ag-Cd	.25	.03	42	13			
Low group:							
Qc-Qwf	.21	.04	50	7			
Qc	.20	.02	62	40			
Qwf	.18	.03	38	7			
Pm	.16	.02	24	4	Low		
Af	.06	------	------	1			
Group means:							
High	.26	.02	47	42	s		
Low	.19	.02	59	59			
All types	.22	.01	55	101			

[1] For meaning of abbreviations see footnotes to tables 1 and 2.

Because the types with the larger amounts of forest floor tend to have lower field moisture capacities and vice versa, the figures for depth of water retained, which integrate the two, would be expected to be less definitely differentiated. This proved to be the case. In

Bell Canyon the range of the means of the different types is from 0.04 to 0.21 inch. Only the mixed type of chamiso (Af) and *Ceanothus crassifolius*, and in one case the black sage (Sm)–Eriogonum mixture less common types are significantly different from certain of the black sage (Sm) mixtures.

When the types in Bell Canyon are grouped, a high group of seven types is found to be identical with the high group that was distinguished on the basis of dry weights of forest floor. The other seven types, including those with chamiso (Af) and *Eriogonum*, comprise a rather homogeneous group, characterized by low values for depth of water retained. The mean for the high group is 0.12 inch and for the low group 0.06 inch, and these two groups differ one from another with high significance.

The analysis shows somewhat similar results in Fern Canyon, except that the depth of water retained by the different types is greater with only two exceptions. The range, excluding a single sample of chamiso, is from 0.16 to 0.29 inch. The individual types are not significantly different one from another.

When the types in Fern Canyon are grouped, those with low values for mean depth of water retained form the same group of five types without manzanita which comprised the low group for dry weight of forest floor. The other six types containing manzanita (Ag) form a group with high values. The mean for this high group is 0.26 inch and for the low group 0.19, and the two groups are significantly different one from another.

There is almost no overlapping in the mean values of individual types between Bell and Fern Canyons, and the means for the respective watersheds are significantly different.

These figures for depth of water retained at field moisture capacity on the basis of oven-dry weight have special interest because they represent the depth of precipitation which would be retained by the forest floor, subject to evaporation into the air, and which would therefore not be available either for the growth of vegetation or for replenishing soil moisture or ground water. However, to use them in this way, a correction must be made because the forest floor under natural conditions is never reduced to an oven-dry condition. During dry weather the floor usually contains about 15 percent moisture content on the basis of oven-dry weight. Hence the figures for field moisture capacity in percentage would be reduced by 15 percent, or by the percentage of actual moisture content at the beginning of any rain, and the figures for depth of water retained could be reduced correspondingly. In light rains the forest floor may be a factor of some importance in intercepting moisture which would otherwise reach the soil. In heavy rains, on the other hand, it will affect only a negligible part of the total rainfall.

SUMMARY

The forest floor under the chaparral covers a large part of the surface within 15 years after a heavy burn.

The amount of forest floor varies widely within any given type of chaparral, although differences in means between types are small and only significant between the extremes. The differences become sig-

nificant if the chaparral is segregated into only two or three broad groups such as those characterized by chamiso (Af), manzanita (Ag), or oak (Qc, Qwf). The 15 metric tons average for Fern Canyon is almost three times that for Bell Canyon.

The mean volume weights for individual types vary from less than 0.1 to 0.36. In Fern Canyon there is a well-defined differentiation between the types or group with high means in which manzanita, chamiso, or bigcone spruce (Pm) predominate, and those with low means in which the oaks predominate.

Field moisture capacities vary less within types than the other measures. Three groups within each canyon show a significant difference in the means, although the difference between 157 percent in one canyon and 150 percent in the other is not significant. In general the manzanita, *Ceanothus*, and chamiso are associated with low field moisture capacities of the floor, and oak and big cone spruce with high.

The depth of water retained by the forest floor represents an integration of amount, volume weight, and field moisture capacity of the materials, and gives an indication of the role of the forest floor in intercepting rainfall. Like its component elements, it varies within types to such an extent that the small differences between the means of types are rarely significant. The groups of types, however, are distinct, a low group in Bell Canyon retaining 0.06 inch, a high group, 0.12 inch; and in Fern Canyon, a low group, 0.19, and a high group, 0.26 inch. These amounts, less the actual moisture content at the beginning of rain, would be held subject to evaporation from the forest floor and would not contribute to soil moisture or to surface run-off.

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THE ANNUAL ACCUMULATION AND CREEP OF LITTER AND OTHER SURFACE MATERIALS IN THE CHAPARRAL OF THE SAN GABRIEL MOUNTAINS, CALIF.[1]

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The amount of organic material which accumulates each year beneath the chaparral in southern California constitutes one factor in the development of the forest floor, or A_0 horizon; and to the extent that it is composed of leaves, it may be an index of the transpiration of water by the vegetation. The annual accumulation has been collected each year for 1935, 1936, and 1937 from the "triplicate" watersheds at the heads of Bell and Fern Canyons in the San Dimas Experimental Forest of the San Gabriel Mountains.[2] The areas have been described in general by the California Forest Experiment Station,[3] and more specifically in a study of the total forest floor.[4]

It is only necessary to recall here that the slopes are steep, from 10° to 50°, and that the chaparral in Bell Canyon, at elevations between 2,500 and 3,500 feet, has come in since the severe fire of 1919, whereas that in Fern Canyon, at elevations from 4,500 to 5,400 feet, has been undisturbed for more than 50 years. The annual accumulations have been analyzed according to communities characterized by the predominating species of chaparral. Nine types are represented in Bell Canyon and 10 in Fern.

The collections were made by placing trays 3 feet square, with wooden edges 2 inches high, and 40-mesh screen wire for the bottoms, alternately 2 rods above and below the contour trails at the representative points where the rain gages were located by the Forest Experiment Station. The 64 trays in Bell Canyon and 54 in Fern may thus be considered to be representative of the variations in the vegetation in the two canyons, and perhaps by inference over a wider area. The material in the trays has been collected in late April in each of the 3 years. For various reasons, collections were not made from all the trays each year, so that the actual records comprise less than three times the number of trays.

Frequently there was evidence that organic and mineral material had entered the trays from above by creep or washing, or that some material had been lost at the lower edge. All such samples were excluded from the analysis. However, even when no creep was evident, the material often contained some mineral matter, and consequently all of the samples were separated from any mineral matter by a process of sieving and floating in the laboratory. The oven-dry

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[2] The work was made possible by the allotment of funds from a special State appropriation for research in forestry in cooperation with Federal agencies, and has been carried on with the cordial cooperation of the California Forest Experiment Station.

[3] United States Forest Service, California Forest and Range Experiment Station. THE SAN DIMAS EXPERIMENTAL FOREST. 19 pp., illus. Berkeley, Calif. Revised, 1936.

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weights were then determined and converted to metric tons per acre. An attempt was made with the 1935 collections to separate the leaf material from the rest of the accumulations in order to learn what percentage the leaves made of the total and also their amount in dry weight per acre.[5] These data, the relations between the individual years, and the ratio of the annual accumulation to the total forest floor are shown in table 1.

TABLE 1.—*Annual accumulation of litter, in chaparral in Bell and Fern Canyons, 1935–37*

BELL CANYON

Chaparral type [1]	Average annual accumulation per acre [2]	Samples	Each year as percentage of 3-year average			Average total floor per acre [2]	Ratio of total to annual accumulation	Leaves only		
			1935	1936	1937			Samples	Amount per acre [2]	Percentage of 1935 annual accumulation
	Metric tons	*Number*	*Percent*	*Percent*	*Percent*	*Metric tons*		*Number*	*Metric tons*	*Percent*
Ag	1.85	2	88	112	....	4.13	2.2	1	1.08	66.7
Ccr-Af	.79	23	80	163	100	5.17	6.5	12	.29	47.9
Af-Ccr	.76	27	64	154	84	4.21	5.5	8	.28	49.4
Sm-Ccr	.67	7	72	170	57	7.40	11.0	2	.29	52.8
Qd-Ccr	.63	12	68	155	97	3.75	6.0	3	.17	54.1
Ccr-Sm	.54	13	80	146	120	6.72	12.4	4	.30	62.0
Gv	.49	5	43	133	155	9.50	19.4	1	.20	54.6
Af-Sm	.37	13	59	130	93	4.82	13.0	3	.01	5.6
Sm-Eff	.34	8	94	77	126	2.92	8.6	3	.19	61.0
All types	.64	110	71	145	[3] 91	5.31	8.4	37	.27	53.0

FERN CANYON

Chaparral type [1]	Average annual accumulation per acre [2]	Samples	1935	1936	1937	Average total floor per acre [2]	Ratio of total to annual accumulation	Samples	Amount per acre [2]	Percentage of 1935 annual accumulation
Ag-Af	1.40	3	65	124	110	20.56	14.7	1	0.40	44.5
Ag-Cd	1.08	4	88	104	102	20.16	18.7	1	.41	43.1
Ag	.98	13	81	116	108	21.18	21.6	4	.51	44.6
Qw-Ag	.90	2	84	....	117	17.57	19.5	2	.27	39.0
Qc-Qwf	.60	9	73	122	107	12.84	21.4	2	.30	56.6
Qwf	.53	8	57	113	96	12.29	23.2	4	.22	43.5
Cd-Ag	.48	5	69	98	183	19.74	41.1	2	.16	47.7
Qc	.42	56	95	127	98	12.24	29.1	14	.16	44.9
Pm	.20	3	100	115	80	10.11	50.5	1	.07	36.9
Af	.19	3	69	126	111	4.69	24.7	1	.001	.7
All types	.57	106	83	[4] 116	100	15.06	26.5	32	.23	44.4

[1] Abbreviations here used: Af, *Adenostoma fasciculatum*, chamiso; Ag, *Arctostaphylos glauca*, manzanita; Qc, *Quercus chrysolepis*, canyon live oak; Ccr, *Ceanothus crassifolius*; Cd, *C. divaricatus*; Eff, *Eriogonum fasciculatum* var. *foliolosum*, wild buckwheat; Gv, *Garrya veatchii*, silktassel; Qd, *Quercus dumosa*, scrub oak; Qwf, *Q. wislizenii* var. *frutescens*, interior liveoak; Sm, *Salvia mellifera*, black sage; Pm, *Pseudotsuga macrocarpa*, bigcone-spruce.

[2] Metric tons oven-dry weight after separation of mineral matter.

[3] Based on 8 types.

[4] Based on 9 types.

The average annual accumulation for the different chaparral types varied from 0.19 metric ton per acre for chamiso (Af) up to 1.85 for manzanita (Ag). In a general way, those types that contained manzanita or *Ceanothus* tended to have heavier annual accumulations than the types characterized by chamiso black sage (Sm), false buckwheat (Eff), oak (Qd, Qwf, or Qc), or bigcone-spruce (Pm). The types in the table are arranged in descending order of the average annual accumulation for each canyon. It is at once evident that the differences between adjacent types are not large, although the highest is three to five times the lowest. When all of the types for each canyon are combined in a single average, the annual accumulation for Bell Canyon was 0.64 and for Fern Canyon 0.57 metric ton per acre.

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[5] The help of J. E. Aschoff, W. E. Davis, E. D. Marshall, and J. Vlamis in this work is gratefully acknowledged.

The amount of the annual accumulation varied from year to year for the same sampling points. This might be expected from the well-known variation between seasons as to their favorableness for the growth and development of the vegetation. In the case of the evergreen species, however, it is not the previous season but one of several years earlier in which the reason for a light or heavy fall must be sought. With few exceptions, all of the types in each canyon showed the lowest accumulation in 1935, the highest in 1936, and intermediate amounts in 1937. These figures, expressed as percentages of each year to the 3-year averages, are shown in the table for each of the chaparral types. In individual communities the spread between the different years may be considerable, as for example in the chamiso-*Ceanothus* (Af–Ccr) in Bell Canyon, where the 1935 accumulation constituted 64 percent, the 1936, 154 percent, and the 1937, 84 percent of the 3-year average for the type. Combining all types for Bell Canyon, the average percentages were 71, 145, and 91; and for Fern Canyon 83, 116, and 100 for 1935, 1936, and 1937 respectively. Both for the individual types and for the averages, the variation between years was less marked in Fern Canyon than in Bell Canyon.

When the figures for the annual accumulation are divided into those for the total forest floor, a series of ratios is obtained which represents the relation between the total and the annual accumulations. The sampling points for total forest floor were not identical with those for the annual accumulations, although both were mechanically spaced over the same area. For that reason, the ratios are more reliable for the types with larger numbers of samples than for those with fewer samples. These ratios also indicate the number of years of annual accumulation which would be required to yield the totals if accumulation were the only process involved. Of course, actually the processes of decomposition are going on in the forest floor each year, so that the ratio of total to annual should be less than the number of years since the last severe fire. In general this was the case. The ratios for Bell Canyon ranged from 2.2 to 19.4, with an average of 8.4 where the San Gabriel fire occurred from 16 to 18 years prior to the years of collection. In Fern Canyon, on the other hand, the ratios ranged from 14.7 to 50.5 with an average of 26.5 where the vegetation had been undisturbed for more than 50 years. The relation between this ratio and either the total forest floor or the annual accumulation alone is not well defined in Bell Canyon; but in Fern, the types with high accumulation and larger amounts of forest floor, namely, those characterized by manzanita (Ag), tend to have low ratios, whereas the canyon live oak (Qc) type has a rather high ratio. Because of the many factors that influence the rate of decomposition of the forest floor, it is readily apparent that this ratio, in which rate of decomposition is an important factor, may vary widely with types of vegetation, or with small differences in site.

The annual accumulation of leaf material expressed as a percentage of the total accumulation, and in metric tons per acre, is given for the 1935 season. In Bell Canyon the leaves constituted from 47.9 to

66.7 percent of the annual accumulation, with one exception. The average was 53 percent. In Fern Canyon with one exception the percentages ran a little lower, from 36.9 to 56.6 percent, with an average of 44.4 percent. In both canyons the types characterized by chamiso (Af) formed the exceptions, with percentages of 0.7 and 5.6.

The dry weights per acre of leaf material ranged from less than 0.01 to more than 1.00 metric ton per acre, with an average of 0.27 for Bell Canyon and 0.23 for Fern Canyon. Again the chamiso type forms exceptions, with very low weights of leaf material. Among the other types, the only noticeable tendency is for the heavier amounts of leaf material to be associated with the types containing manzanita (Ag). These are also the types which tend to have the heavier amounts of forest floor.

In both the total annual accumulation and the dry weight of leaf material, the averages for the two canyons are surprisingly close, considering the greater size and apparent luxuriance of the long-undisturbed types of Fern Canyon.

The collections which have been excluded in the foregoing analysis, because of creep of mineral and other material from above over the 2-inch upper side of the tray, provide incidentally an indication of the prevalence of this kind of soil movement on the slopes. When the trays are placed, the upper edge constitutes a barrier to creep, 2 inches or a little more in height above the soil surface, and roughly perpendicular to the slope. When material fills the resulting wedge of space above the tray to such an extent that some of it spills over into the tray, an appreciable movement of soil is indicated. The trays in Bell and in Fern Canyons for each of the years 1935, 1936, 1937, provided a record of the prevalence of creep of this magnitude on these two watersheds. Because the trays were mechanically distributed, the proportion of them which were affected by creep should be roughly indicative of the proportion of area which was subject to soil movement of similar character and intensity. However, in making this application to area, a correction is necessary because the creep is effective over only a part of the 3-foot length of the upper edge of the tray. Measurements of the actual distance subject to creep made first in 1938, indicate for Bell Canyon, an average of 1.2 feet, or 40 percent of the 3-foot length. For the 3 years in Bell Canyon with 164 records as a basis, 23 percent of the trays showed considerable creep, 18 percent showed a little, and the remaining 59 percent none. In other words, the creep in Bell Canyon each year is sufficient to override the 2-inch barrier of 41 percent of the trays. Applying the correction of 40 percent of the length, 16 percent of the area is probably subject to creep.

In Fern Canyon there was marked creep in 17 percent of the trays, a little in 13 percent, and none in the remaining 70 percent, on the basis of 150 records. The actual creep covers 1.8 out of 3 feet, or 60 percent on an average. Then, in this canyon with the older undisturbed vegetation, creep is indicated for 30 percent of the trays, and for 60 percent times 30, or 18 percent of the area, as compared with 16 percent in Bell Canyon.

The occurrence of creep was analyzed in more detail in relation to the degree of slope. In Bell Canyon the trays in which creep was recorded had the same average slope—35°—as those showing no creep. In Fern Canyon, on the other hand, the marked creep occurred in trays on an average slope of 36°, a slight creep in those of 33°, and no creep in those of 28°. In general, it seems to be the location of individual trays with respect to local peculiarities of surface or of animal activity, rather than the systematic variation in degree of slope that affects the creep.

SUMMARY

The results of the study may be summarized in the following statements.

The average annual accumulation of organic material in the chaparral communities of diverse composition, age, and location rarely varied beyond the amounts of 0.2 to 1.4 metric tons per acre, with a general average of 0.6.

In different years, the annual accumulation varied from less than 50 to over 150 percent of the 3-year average.

The ratio of total forest floor to the annual accumulation increased with age of the vegetation from about 8 in the 18-year-old chaparral of Bell Canyon to over 26 in the more than 50-year-old types of Fern Canyon.

Leaf material constituted from 37 to 67 percent of the annual accumulation and amounted to about 0.25 metric ton per acre on an average. Two exceptions, with percentages of 0.7 and 5.6, may be mentioned for types to which chamiso gave character.

Sufficient creep of loose surface material took place annually to override a 2-inch barrier on 18 percent of the area of Fern Canyon and on 16 percent of the area of Bell Canyon.

A FURTHER STUDY OF THE PRODUCTION OF MILK OF ABNORMAL COMPOSITION BY COWS FREE FROM UDDER STREPTOCOCCI[1]

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INTRODUCTION

The results of a study of the composition of the first 30 cc of milk drawn from each quarter of the udders of 31 cows during their first lactation period were presented in an earlier report.[2] In some of the animals the milk conformed so closely to accepted standards for chlorine content and for pH and catalase values as to indicate that the mammary glands were normal in all respects. In other animals the deviations from the usual standards were so marked and persisted so long as to indicate an abnormal condition in one or more quarters of the gland, and in still others the deviations were so slight or so inconstant as to make difficult the assignment of the animal to a normal or an abnormal group. The deviations in composition were such as would be expected when some part of the gland was inflamed. The foremilks were more alkaline, lower in titratable acidity, slower to coagulate with rennet, and higher in catalase and chlorine than foremilk that is considered normal.

Since bacteria and especially certain streptococci are believed responsible for inflammation of the udder, much effort was made to obtain a quantitative and qualitative picture of the micro-organisms in the separate quarters of each animal throughout the lactation period. For this purpose various culture methods were used.

The findings did not conform to expectation in the case of the animals whose mammary glands showed evidences of inflammation at some level of intensity, for no streptococci were found and the abnormalities in the composition of the milk could be related to no definite qualitative or quantitative deviation in the flora of the udder. Nevertheless if the deviation of the milk from recognized standards is accepted as evidence of inflammation of the udder, a large part of the group would have been considered unfit for the production of milk for human consumption.

Some of the animals were retained in the herd during the second lactation period and observations similar to those made in the first period were continued. The results are presented in the present paper.

EXPERIMENTAL PROCEDURE

The term "normal" refers to an animal whose foremilk from each quarter conformed to two of the following criteria: A pH value below 6.8, a catalase value below 50, and a chlorine content below 0.15 percent. The methods of analyses were described in the previous

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paper. The use of more than one criterion is necessary since animals vary and it is possible to have the milk of a normal animal fail to conform to one of the above criteria, but probably not to two except in the colostric period or very late in the lactation period. The criteria for chlorine and pH are probably reasonably correct; that for catalase is undoubtedly too high since the milk of many animals does not at any time reach this value. It is believed that the above criteria when applied to a considerable number of samples taken at intervals throughout a lactation period offer a reasonably safe means of grouping animals on the basis of condition of the udder. It seems probable that nearly all animals will show abnormalities in the milk from time to time, and that such transitory deviations from the usual condition are of little importance.

Twelve of the thirty-one animals, whose records were presented in the previous report were studied in their second lactation period and also one animal not included in the earlier experiments. For purposes of discussion, the 13 animals are placed in four groups as follows:

Group 1. Cows 1, 7, and 8, which were normal in both the first and second period.

Group 2. Cow 6, which was normal in the first and abnormal in the second period.

Group 3. Cows 22 and 32, which were questionably normal in the first and abnormal in the second period.

Group 4. Cows 5, 11, 15, 21, 31, 34, and 39, which were abnormal in each period.

The herd was maintained for the purpose of studying certain phases of pasture management, and the animals to be retained in the herd during the second period were selected primarily with regard to time of freshening, the condition of the udder noted in the first period being a secondary consideration. This explains why a much larger number of the animals classed as abnormal in the first period were retained than of those classed as normal.

The usual sample consisted of the first 30 cc of milk from each quarter. At less frequent intervals the remainder of the milk produced by each quarter was examined in greater detail. The former are referred to as "routine," the latter as "special" samples.

The record of the animals normal in each period is presented for both the first and the second periods, but for the remainder only such reference is made to the first period as is necessary to give the reader a reasonably complete picture of the animal during the entire period of observation. The record of the normal animals serves as a standard for comparison.

ANIMALS NORMAL IN THE FIRST AND SECOND LACTATION PERIODS

Cow 1 was observed during 245 days in the first lactation period, in which 17 routine samples were taken from each quarter, and during 254 days in the second period, in which 9 routine samples were secured from each quarter. In each period the last sampling was made about 1 month before the animal was dried off. The data of table 1 present the picture of a cow producing milk that conforms closely to accepted standards. The normality of the animal was reflected by the normal condition of the udder at slaughter, 1 month after the last observation was made.

Cow 7 was observed 285 days in the first period, in which 30 routine samples were collected from each quarter, and in 302 days of the second lactation period, in which 10 samples were collected. The final sampling was made about 1 month before the end of each period. The detailed data in table 1 are those of a normal animal. The maximum percent of chlorine, 0.193, may attract attention. Since the values noted 7 days before the one on which the maximum value was found and the one 7 days thereafter were normal, and since there was no disturbance in the catalase or in the reaction of the milk, this maximum value is considered to have no significance.

Cow 8 presents a picture of normality in the case of the 32 samples taken from each quarter in the first 322 days of the first period and the 8 samples in the 274 days of the second. About 12 pounds of milk were produced per day at the date of last sampling in the first period, about 1 month before drying off. Table 1 shows the values for chlorine and catalase to be normal. The maximum values for chlorine, 0.211, and for catalase, 58, are not significant for the reasons presented in the discussion of cow 7. The data for the samples taken 1 month before the end of the second period are not presented. They were abnormal with respect to chlorine and catalase. Thus the chlorine content of the milk from the quarters was 0.276, 0.224, 0.252, and 0.248 percent, respectively. The daily production at that time was 12 pounds per day. It seems certain that in the first period there was no particular change in the composition of the milk as the end of the lactation period approached, but at the end of the second period, marked changes occurred. It is thus evident that an animal reacts differently in different periods as regards the change in the composition of the milk as production decreases.

TABLE 1.—*Percentage of chlorine and of catalase in the milk of cows giving normal milk in the first and second lactation periods*

Cow and quarter	First lactation period				Second lactation period			
	Minimum chlorine	Maximum chlorine	Minimum catalase	Maximum catalase	Minimum chlorine	Maximum chlorine	Minimum catalase	Maximum catalase
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Cow No 1.								
1	0 100	0.155	2	29	0.117	0 161	4	49
2	107	.144	2	22	.111	.134	3	20
3	.111	.141	5	28	.111	.127	3	32
4	.107	.158	5	31	.100	.141	5	35
Cow No 7								
1	.117	.193	0	20	.104	.138	5	18
2	.104	.141	0	20	.104	.138	5	24
3	.121	.144	10	75	.100	.127	5	26
4	.111	.141	0	31	.104	.138	3	58
Cow No. 8.								
1	.134	.182	0	16	.111	.185	3	22
2	.127	.148	0	22	.117	.211	6	58
3	.127	.155	0	22	.121	.175	4	36
4	.124	.161	0	28	.111	.178	5	23

One set of samples obtained early in the third period was normal in composition.

AN ANIMAL NORMAL IN THE FIRST LACTATION PERIOD AND BECOMING ABNORMAL IN THE SECOND

The data for the first lactation period of cow 6 are presented in table 2. The period of observation was 307 days in length and 33 samplings were made. At one sampling the chlorine content of each quarter was high, being 0.217, 0.182, 0.255, and 0.178 percent, respectively, for the quarters. The chlorine values 7 days earlier and 7 days later were normal, as were the catalase values on the specific date. The abnormal values were, therefore, either erroneous or indicative of a transitory condition and were not included in table 2.

TABLE 2.—*Percentage of chlorine and catalase in the milk of cow No. 6 which gave normal milk in the first but abnormal in the second lactation period*

Quarter	First lactation period				Second lactation period			
	Minimum chlorine	Maximum chlorine	Minimum catalase	Maximum catalase	Minimum chlorine	Maximum chlorine	Minimum catalase	Maximum catalase
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	0.124	0 175	0	36	0.111	0.193	6	90
2	117	.148	0	34	.107	.303	5	254
3	.121	.144	6	41	104	.155	6	66
4	.121	.144	3	48	.107	.172	4	54

Ten sets of samples were obtained during 302 days of the second period. The data in table 3 are presented to show the gradual increase in abnormality in the last third of the second period. Each quarter is involved in a change for which no reason was evident.

TABLE 3.—*Percentage of chlorine and catalase in the milk of cow No. 6 on certain days in the last third of the second lactation period*

Days since freshening	Quarter 1		Quarter 2		Quarter 3		Quarter 4	
	Chlorine	Catalase	Chlorine	Catalase	Chlorine	Catalase	Chlorine	Catalase
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
208	0 193	90	0 127	14	0.131	20	0 148	18
237	.155	27	.185	62	.138	19	.138	22
274	.158	54	224	254	.127	66	.148	54
302	.189	80	.303	190	.155	40	.172	36

ANIMALS QUESTIONABLY NORMAL IN THE FIRST LACTATION PERIOD AND ABNORMAL IN THE SECOND

In the case of cow 32 the abnormality during the first period was confined to quarter 2. Of the 16 samples taken from the one hundred and fifth to the three hundred and ninth day, 4 were abnormal and the remainder differed sufficiently from those collected earlier in the period to indicate that a decided change in the quarter had occurred. The data for the routine samples are shown in table 4, and for the special samples of the second period in table 5. It is to be noted that quarter 3 was normal in each period and that as the second period passed, the percentage of milk from this quarter constantly increased. The animal produced 260 pounds less milk in the same number of days (243) in the second period than in the first. It seems probable that the gradually increasing abnormality in the other quarters was responsible for the decrease in the production of these quarters.

TABLE 4 — *Percentage of chlorine and of catalase in the milk of cow No. 32 which gave questionably normal milk in the first and abnormal in the second lactation period*

Quarter	First lactation period					Second lactation period				
	Minimum chlorine	Maximum chlorine	Average chlorine	Minimum catalase	Maximum catalase	Minimum chlorine	Maximum chlorine	Average chlorine	Minimum catalase	Maximum catalase
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	0. 114	0. 144	0 125	0	57	0 138	0 168	0 145	16	264
2	. 114	239	172	10	233	. 131	185	161	16	230
3	111	. 155	. 124	0	49	. 107	. 165	. 121	9	78
4	. 114	. 144	. 133	4	108	. 097	. 189	. 152	9	400

TABLE 5.— *Yield, catalase, and chlorine content, and acidity of both the foremilk and the remainder of the milk (whole milk) from the different quarters of cows giving abnormal milk, sampled at various times after lactation began*

COW NO 32

Days since freshening	Quarter	Yield		Catalase		Chlorine		Acidity	
				Foremilk	Whole milk	Foremilk	Whole milk	Foremilk	Whole milk
		Pounds	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
37	1	4 7	19	196	99	0 161	0 127	0 117	0. 144
	2	7 2	27	296	306	155	138	126	. 162
	3	7 2	27	31	19	. 114	. 097	. 162	. 162
	4	7 0	27	34	19	104	. 100	180	. 189
Total		26 1							
85	1	2 4	14	32	12	138	. 093		
	2	4 1	25	50	47	138	117		
	3	5 2	32	44	25	. 131	. 107		
	4	4 6	29	5	3	097	. 100		
Total		16 3							
163	1	2 0	15	44	16	200	. 161	. 153	184
	2	3 5	25	61	65	. 197	. 168	. 153	. 180
	3	4 7	34	6	8	. 155	. 138	. 180	. 189
	4	3 6	26	220	224	. 197	. 178	. 180	. 171
Total		13 8							
218	1	1. 0	15	33	36	. 144	. 114	. 162	. 189
	2	1 0	15	79	28	. 152	. 127	. 153	. 180
	3	2 8	42	19	18	. 117	104	. 180	. 198
	4	1 8	28	228	255	. 185	141	. 126	. 171
Total		6 6							

COW NO. 22, FIRST LACTATION PERIOD

Days since freshening	Quarter	Yield		Catalase		Chlorine		Acidity	
		Pounds	*Percent*	Foremilk	Whole milk	Foremilk	Whole milk	Foremilk	Whole milk
153	1	2 0	21	10	21	0. 117	0. 111	0. 153	0. 157
	2	3. 1	33	15	7	. 124	. 114	. 162	. 157
	3	2. 6	28	10	4	. 121	. 117	. 153	153
	4	1 7	18	76	38	138	138	153	157
Total		9. 4							
161	1			13	13	. 152	. 127	130	. 162
	2			14	22	. 165	155		. 162
	3			17	22	148	. 117		. 153
	4			56	46	. 178	. 131	. 108	. 153
183	1	3. 1	19	74		. 178		. 108	
	2	4 8	30	10	16	. 144	. 131	. 126	. 153
	3	5 3	33	5	6	. 127	114	. 144	. 162
	4	2. 8	18	20	26	152	127	. 126	. 144
Total		16. 0							

TABLE 5.—*Yield, catalase, and chlorine content and acidity of both the foremilk and the remainder of the milk (whole milk) from the different quarters of cows giving abnormal milk, sampled at various times after lactation began*—Continued

COW NO. 22, FIRST LACTATION PERIOD—Continued

Days since freshening	Quarter	Yield		Catalase		Chlorine		Acidity	
				Fore-milk	Whole milk	Fore-milk	Whole milk	Fore-milk	Whole milk
		Pounds	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
219	1	3 2	18	34	27	.155	.124	.153	.162
	2	5 7	32	70	29	.134	.134	--------	.162
	3	6 1	34	17	10	.131	.117	.153	.180
	4	2.9	16	70	33	.144	.127	.153	.162
Total		17.9							

COW NO. 22, SECOND LACTATION PERIOD

Days since freshening	Quarter	Yield		Catalase		Chlorine		Acidity	
				Fore-milk	Whole milk	Fore-milk	Whole milk	Fore-milk	Whole milk
		Pounds	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
6	1	5 2	23	200	126	0 214	0 138	0 099	0.171
	2	8.5	39	210	107	.214	.131	.081	189
	3	2 0	29	264	242	.276	.155	.108	171
	4	6 5	29	47	45	.148	.121	.144	189
Total		22 2							
39	1	7 0	26	23	18	.144	.117	.126	.153
	2	10 2	38	14	7	127	.111	.144	.172
	3	3 2	12	188	82	.221	.141	.063	.126
	4	6.2	24	8	11	.111	.107	.162	.162
Total		26 6							
83	1	5.0	24	27	19	155	.124	.108	144
	2	8 1	39	18	12	.148	.117	.117	.144
	3	2.1	10	70	22	.172	.117	.104	.135
	4	5.6	27	5	9	.121	114	.139	.153
Total		20 8							
80	1	2 4	22	240	214	.296	.158	.045	130
	2	5 2	47	21	12	148	114	.121	162
	3	7	6	85	117	.200	.117	.090	.153
	4	2 7	25	24	24	.161	.117	.108	.153
Total		11 0							
213	1	2 4	16	198	120	.255	.138	.072	144
	2	7.2	49	35	45	.148	.117	.121	.153
	3	1.2	8	88	48	.200	121	.081	153
	4	3 9	27	36	20	.152	111	.126	.153
Total		14 7							

COW NO 5, SECOND LACTATION PERIOD

Days since freshening	Quarter	Yield		Catalase		Chlorine		Acidity	
				Fore-milk	Whole milk	Fore-milk	Whole milk	Fore-milk	Whole milk
		Pounds	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
27	1	3.6	19	4	7	0 134	0 131	0.135	0.144
	2	5.5	30	13	9	.148	.131	--------	.144
	3	4 8	26	30	30	.158	.134	.117	.126
	4	4.7	25	16	8	.127	131	139	.130
Total		18 6							
31	1	5.1	24	9	20	127	.124	.144	.135
	2	5 6	26	13	16	.138	.117	.126	.135
	3	5 5	25	13	12	.134	.114	.117	.135
	4	5 5	25	7	12	.124	.117	.126	.135
Total		21 7							
96	1	3.2	22	238	42	.197	.144	.108	.122
	2	3.7	25	140	51	.217	.155	.099	.126
	3	4.0	28	150	37	.203	.155	.090	.126
	4	3.7	25	64	27	.182	.138	.108	.135
Total		14.6							
231	1	3.7	25	85	22	.152	.148	.135	.135
	2	3.8	26	83	18	.152	.144	.144	.135
	3	3.8	26	107	22	.144	.141	.144	.144
	4	3 6	23	51	13	.144	.138	.144	.135
Total		14.9							

TABLE 5.—*Yield, catalase, and chlorine content, and acidity of both the foremilk and the remainder of the milk (whole milk) from the different quarters of cows giving abnormal milk, sampled at various times after lactation began*—Continued

COW NO 11, SECOND LACTATION PERIOD

Days since freshening	Quarter	Yield		Catalase		Chlorine		Acidity	
				Fore-milk	Whole milk	Fore-milk	Whole milk	Fore-milk	Whole milk
		Pounds	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
31	1	5.5	23	22	53	0.141	0.144	0.144	0.144
	2	.5	2	290	261	.303	.211	.054	.090
	3	10.9	46	3	5	.144	.138	.162	.162
	4	6.6	29	6	5	.141	.141	.162	.153
Total		23.5							
75	1	4.6	25	54	146	.165	.152	.135	.126
	2	.4	2	198	196	.248	.211	.063	.090
	3	7.9	43	5	7	.148	.144	.127	.126
	4	5.6	30	4	8	.148	.141	.135	.126
Total		18.5							
144	1	4.4	31	101	102	.203	.182	.099	.126
	2	.4	3	183	98	.293	.231	.054	.072
	3	7.6	55	16	14	.168	.152	.135	.144
	4	1.6	11	96	45	.185	.175	.117	.117
Total		14.0							
171	1	3.0	29	135	99	.221	.158	.081	.121
	2	.3	3	162	216	.262	.224	.063	.090
	3	6.2	59	16	16	.158	.138	.135	.144
	4	1.0	9	79	58	.189	.158	.099	.126
Total		10.5							
185	1	1.5	24	128	81	.237	.149		
	2	.1	2	158	187	.301	.237		
	3	4.0	65	14	19	.165	.145		
	4	.5	9		59	.207	.166		
Total		6.1							
COW NO 15, SECOND LACTATION PERIOD									
28	1	6.8	19	31	11	0.172	0.158	0.117	0.144
	2	10.7	30	13	6	.189	.158	.112	.135
	3	11.7	33	19	30	.175	.172	.126	.126
	4	6.5	18	4	26	.158	.158	.148	.144
Total		35.7							
64	1	4.5	19	66	24	.214	.172	.099	.117
	2	8.1	33	6	3	.211	.178	.090	.117
	3	7.4	30	14	13	.207	.172	.108	.108
	4	4.2	18	10	5	.182	.168	.135	.126
Total		24.2							
161	1	2.8	15	191	189	.248	.203		
	2	6.2	32	67	100	.200	.197		
	3	6.3	33	41	75	.182	.203		
	4	3.7	20	59	77	.175	.193		
Total		19.0							
COW NO. 21, SECOND LACTATION PERIOD									
13	1	7.1	27	11	13	0.127	0.141	0.180	0.171
	2	6.8	25	74	157	.158	.148	.144	.162
	3	7.4	28	18	42	.134	.131	.180	.171
	4	5.4	20	19	59	.148	.127	.171	.162
Total		26.7							

TABLE 5.—*Yield, catalase, and chlorine content, and acidity of both the foremilk and the remainder of the milk (whole milk) from the different quarters of cows giving abnormal milk, sampled at various times after lactation began*—Continued

COW NO 21, SECOND LACTATION PERIOD—Continued

Days since freshening	Quarter	Yield		Catalase		Chlorine		Acidity	
				Fore-milk	Whole milk	Fore-milk	Whole milk	Fore-milk	Whole milk
		Pounds	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
54	1	8.5	34	4	7	0.121	0 111	0.153	0.162
	2	4.1	16	137	199	.182	.175	.117	.117
	3	6.2	24	6	9	.117	121	162	.162
	4	6.6	26	19	47	.117	124	.166	157
Total	....	25 4	....	....	....	....	....	....	....
78	1	7.9	32	3	31	.121	.114	.162	.162
	2	4.3	17	238	146	.282	.161	.054	.135
	3	6 5	26	32	33	.189	.131	.099	.139
	4	6.2	25	10	16	.127	.117	.130	.162
Total	....	24 9	....	....	....	....	....	....	....
118	1	4 1	30	12	11	.161	.131	117	.135
	2	2.7	19	227	145	.307	.165	054	108
	3	4.0	29	20	32	138	.144	.135	.126
	4	3 0	22	11	22	.127	134	.135	135
Total	....	13.8	....	....	....	....	....	....	....
177	1	4 6	32	20	12	.117	.121	.135	.144
	2	2 3	16	122	108	.158	.155	.117	126
	3	3.5	24	113	45	.168	.152	.117	.121
	4	3 9	28	39	20	141	.127	.135	.135
Total	....	14.3	....	....	....	....	....	....	....
253	1	3 7	33	30	26	.175	.127	.108	144
	2	2.2	19	204	96	.234	.138	.081	.135
	3	2 5	22	49	27	.193	.134	.108	.144
	4	3 0	26	18	21	.138	.131	....	144
Total	....	11.4	....	....	....	....	....	....	....

COW NO. 31, SECOND LACTATION PERIOD

Days since freshening	Quarter	Yield		Catalase		Chlorine		Acidity	
		Pounds	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
41	1	7.2	28	15	23	0.111	0 104	0.189	0 171
	2	.0	0	282	....	.354	....	....	....
	3	10.1	38	14	18	.114	.111	.162	180
	4	9.0	34	31	6	.104	.104	180	.171
Total	....	26.3	....	....	....	....	....	....	....
77	1	6.9	29	7	5	.114	.111	.135	.162
	2	.0	0	258	....	.351	....	.045	....
	3	10.0	43	5	6	.117	114	.144	.162
	4	6.6	28	12	15	.117	121	.144	.162
Total	....	23.5	....	....	....	....	....	....	....
115	1	7.0	34	30	20	.127	.117	.135	.144
	2	....	....	233	....	.344	....	.036	....
	3	6.3	32	18	26	.131	.121	.126	135
	4	7 0	34	14	78	.127	.138	.144	.126
Total	....	20.3	....	....	....	....	....	....	....
202	1	5 0	37	50	37	.231	.182	.126	.153
	2	.0	0	222	....	.397	....	.063	....
	3	5.0	37	136	133	.252	.197	.108	.162
	4	3.4	26	234	224	.334	.231	.072	.139
Total	....	13.4	....	....	....	....	....	....	....
257	1	2.3	39	106	78	.241	.161	.180	.126
	2	.1	1	227	....	.238	.217	.099	.081
	3	2.3	39	80	123	.234	.168	.081	.135
	4	1.2	21	266	224	.293	.172	.063	.135
Total	....	5.9	....	....	....	....	....	....	....

Cow 22 showed no abnormalities during the first 3 months of the first period, but soon thereafter quarter 4 began to show abnormalities, which continued to the end of the period. Quarter 1 began to show deviations from the normal at a later date. The general nature of the changes is shown in table 5. It should be noted that the changes in the milk were confined to the foremilk; the entire milk from quarters 1 and 4 was not materially different from that of 2 and 3. The yield of the abnormal quarters was maintained as well as that of the normal quarters, and indeed the production of the animal was well maintained in the last part of the period, as the following figures show: Fourth month, 1,058 pounds; fifth month, 982 pounds; sixth month, 824 pounds; seventh month, 832 pounds; and eighth month, 828 pounds. The number of bacteria in the foremilk of quarter 4 was greater in the last part of the period than in the first. The samples from quarter 1, taken during the last 2 months, contained a streptococcus which from a limited study was judged to be of a type not generally believed to be concerned in the production of chronic mastitis.

The dry period was about 2 months in length. Quarter 1 was, in the second period, at first abnormal, then normal, and still later abnormal, while quarter 4 was quite normal during the entire second period. Quarter 3, which had been consistently normal in the first period, was consistently abnormal in the second. Quarter 2 was normal in each period. These changes are reflected in the changes in the relative percentages of the total yield produced by each quarter. In the first period, quarter 3 produced from 28 to 34 percent of the total milk; in the second from 6 to 12 percent. Quarter 4 yielded 16 to 18 percent and 24 to 29 percent in the respective periods. In the second period as in the first the entire milk from the abnormal quarters had much the same composition as that from the normal quarters, but a gradual deterioration of the animal is indicated by a decreased production of milk in the second period.

ANIMALS ABNORMAL IN BOTH THE FIRST AND SECOND LACTATION PERIODS

The remaining seven animals were abnormal in both periods. Cow 34 was observed for 315 days in the first period during which 31 samplings were made, and for 309 days in the second with 12 samplings. The animal was classed as abnormal during the last part of the first period, since the third quarter yielded from the one hundred and eleventh day to the end of the period milks which satisfied the criteria of abnormality. After a dry period of 3 months, the second period began with a normal condition in each quarter. After the two hundred and forty-fourth day, quarters 2 and 3 were definitely abnormal. It is evident from a study of all the data that quarters 2 and 3 began to change from their previous condition by the middle of the first period and did not return to that condition during the remainder of the period of observation.

It is probable that the chlorine content of the foremilk of this animal was about 0.12 to 0.14 percent. The percents of samples of foremilk exceeding these values in the first period were as follows:

First period Quarter:	Percent	*Second period* Quarter:	Percent
1	19	1	20
2	87	2	40
3	100	3	50
4	25	4	33

The case is thus one in which an abnormal condition was established gradually in the rear quarters at the middle of the first period and persisted throughout the second, becoming more marked in the latter part of the second. The productivity was not impaired since the animal produced 8,129 pounds of milk in 308 days of the second period as compared to 5,950 pounds in 311 days of the first period.

Cow 39 was apparently normal during the first 180 days of the observation period of 320 days in the first period. Thereafter quarters 3 and 4 yielded abnormal milk quite consistently to the end of the period. The dry period was about 6 weeks in length. For the first 55 days of the second period, the milk from all quarters was normal. From then on to the end of the period of observation at the two hundred and twenty-third day the milk of every quarter was abnormal. Only foremilk samples were examined in the first period. The more detailed study of the second period showed that the abnormality was confined to the foremilk and that even in the first part of the second period, each quarter was abnormal in some degree. Five thousand four hundred and seventy-seven pounds of milk was produced in 244 days of the first period and 6,759 pounds in 244 days of the second. The increase in production indicates that the abnormality was of low intensity.

The record of the remaining cows, 5, 11, 15, 19, 21, and 31, during the first period was presented in the earlier paper. Only such data will be repeated as are necessary to relate the record of the first period to the second.

Cow 5 was abnormal throughout the first period. The relative condition of the quarters is shown by the percentage of abnormal samples:

Quarter:	Percent
1	15
2	32
3	61
4	3

The record for the second period is shown in table 5. Quarter 4 was relatively free from abnormality during each of the periods. The percentage of milk produced by this quarter ranged from 23 to 25. The low intensity of the abnormality is shown by the relative yields of the different quarters, and by the fact that the entire amount of milk produced by any quarter was rarely of abnormal composition. The composition of the foremilk, especially from quarter 3, would have indicated the removal of the animal even in the first period if indirect tests for inflammation had been relied on.

Cow 11 was observed for 224 days in the first period, which was marked by the consistent abnormality of quarter 2 from the very beginning and the consistent normality of the other three quarters. The animal was observed for 173 days in the second period (table 5). Quarter 2 was essentially nonfunctioning in the second period and quarter 4 became relatively less and less productive toward the end of the period. Quarter 3 was then producing 65 percent of the total yield. The animal was slaughtered soon after the last sampling was made. The post mortem revealed nothing of importance other than had been indicated by the milk.

Cow 15 was in a normal condition at the beginning of the first period, if one can rely on the catalase and pH values. During the early part of the period chlorine was not determined quantitatively, except to learn whether it was above 0.15 percent. The subsequent findings indicated that the normal chlorine value was considerably above this level, for in no instance in the numerous quantitative observations made in each of the two periods was the percentage as low as 0.15, even when catalase and pH values were normal. Quarters 1, 2, and 3 became abnormal within less than 3 months in the first period and remained consistently so to the end of the period on the three hundred and sixth day. Quarter 4 was classed as normal. The dry period was 94 days in length. There was a period of normality at the beginning of the second period, as is shown by the findings on the twenty-eighth day (table 5). One should recognize that chlorine percentages of 0.18 are normal to this animal. By the sixty-fourth day abnormalities were evident and by the one hundred and sixty-first day they were more pronounced. In contrast to the condition in the animals previously discussed, the entire amount of milk produced by one quarter of this animal was abnormal. The later findings, not presented in detail, showed that the abnormality increased in severity as the milking period lengthened. The chlorine content of quarters 1, 2, and 3 was from 0.3 to 0.4 percent.

Cow 21 was abnormal during practically all the first period. The relative condition of the quarters is shown by the following:

Quarter:	*Percent of 30 samples abnormal*
1	3
2	80
3	13
4	20

The relative condition of the quarters was much the same in the second quarter as in the first, as is indicated by the following:

Quarter:	*Percent of 10 samples abnormal*
1	0
2	94
3	25
4	0

The detailed data of cow 21 are presented in table 5. The abnormality of the milk was confined largely to the foremilk so far as acidity and chlorine are concerned; the catalase value of the entire milk remained high.

The second and third quarters of cow 31 became abnormal at about the middle of the first period, which was 379 days long, and the abnormal condition continued to the end of the period. No essential differences were noted between the two quarters. However, in the second lactation (table 5), quarter 2 produced no milk and quarter 3 produced from 32 to 43 percent of the total yield, whereas in the latter part of the first period the percentage was from 13 to 21. This is one of the few instances in which there is reason to believe that improvement in the condition of a quarter occurred.

INFLUENCE OF THE ABNORMALITY ON MILK PRODUCTION

The production of each of the 13 animals in practically an equal number of days in the two lactation periods is presented in table 6. The results are such as might be expected. Each of the animals normal in both periods showed an increase comparable to the expected increase of a second over a first lactation. The animals normal or questionably normal in the first period and abnormal in the second showed decreases in two of the three cases. The animals abnormal in each period gave the most variable results, ranging from a decrease from the production of the first period to an increase of 44 percent. The animals are too few to indicate other than the trend induced by the abnormality. If the 13 are divided into two groups, one group consisting of the animals normal in each period and the other of the remainder, the average increase in production for the first group is 2,151 pounds; for the second 1,053 pounds. Thus the abnormality is seen to have reduced the yield over 1,000 pounds in a lactation period.

TABLE 6.—*Milk-production records of the cows as related to normality or abnormality of the milk*

Cow number	Condition of milk		Yield		Increase (+) or decrease (−) in second period
	First period	Second period	First period	Second period	
			Pounds	*Pounds*	*Pounds*
1	Normal	Normal	7,889	9,991	+2,102
7	do	do	7,353	9,418	+2,065
8	do	do	6,549	8,887	+2,288
6	do	Abnormal	8,989	10,655	+1,666
22 [1]	do	do	10,512	8,902	−1,610
32 [1]	do	do	7,313	7,053	−260
5	Abnormal	do	7,028	10,127	+3,099
11	do	do	5,975	6,178	+203
15	do	do	7,249	8,241	+992
21	do	do	7,145	10,163	+3,018
31	do	do	3,933	3,900	−33
34	do	do	5,950	8,120	+2,179
30	do	do	5,477	6,759	+1,282

[1] Cows 22 and 32 questionably normal in the first period.

DISCUSSION

The record of this isolated herd supports the view that the streptococci causing chronic mastitis are specific inhabitants of the udder and not adventitious forms from some other habitat, such as the digestive tract, and it shows further that it is possible to keep a herd free from them. The production of milk of abnormal composition by one or more quarters over long periods of time means that some agency other than streptococci is responsible for the abnormality.

The animals showed no evidence of abnormality other than that revealed by the periodic examination of the milk. There were practically no swollen udders, and physical changes in the milk were seldom noticed. From the absence of these two conditions the herd would have been classed by the usual herd owner as free from mastitis. If the presence of streptococci is demanded as proof of chronic mastitis, the herd would again have been classed as mastitis-free, since but one animal was shown to harbor streptococci in the udder. In this case the organism was shown to be of a type not supposed to cause mastitis. It produced no disturbance and seemed to be confined to the milk cistern.

In the earlier report, it was stated that no consistent bacterial picture had been noted for the group of cows producing abnormal milk, nor for any one animal. In many instances the number of bacteria was greater in the abnormal foremilk than in the normal foremilk from other quarters of the animal. The exceptions were so numerous, however, that it is not safe to conclude that there was a causal relation between the bacteria that grew in the milk or culture media and the condition of the udder. Instances of a long series of abnormal milk samples from a quarter and a consistently low bacterial content of the foremilk and of the entire milk drawn from the quarter were noted. Again, in a series of consistently abnormal milks, the bacteria might be now high, now low. It may be that the intermittency noted by Johns and Hastings [3] in which a rhythmic variation in the composition of the milk is produced by a rhythmic variation in the number of bacteria, a seesaw between the parasite and the host, existed but was not detectable in the examination since the bacterial and chemical analyses were made on the same sample. It is possible that the examination has not been of the proper nature to reveal significant bacteria, significant because of their number or because of their quality. In the course of the work, numerous media were used and the other factors of the environment were varied. Some of the samples were cultured in litmus milk and on glucose peptone beef-extract agar slopes. Others were plated on veal infusion agar containing horse blood and on glucose agar. The difference in results secured on many samples with the two plating media was so great quantitatively as to indicate that a fancied feeling of safety is easily obtained when only one medium is employed.

After a year's study subsequent to the period represented in the previous report, and with the advantage of the experience acquired, the writers cannot now make a more definite statement as to the cause of the physiological disturbance than was therein made. They can only state as before that no evidence has been secured to indicate that the condition was due to a transmissible agent. The number of animals involved is too small to supply other than suggestive evidence. It would seem that the cause was inherent in the animal, for the four cows that had a normal history in the first lactation period had a normal history in the second, and the nine that were abnormal in the first period were abnormal in the second. Likewise it would appear unwise to conclude, simply on the basis of abnormal chemical reactions of the milk, that a cow is diseased unless other evidence can be secured showing that pathogenic organisms are present in the milk, or that the cow actually has physically detectable symptoms of mastitis. The significance, from the herd owner's standpoint, of the physiological disturbance noted in the animals of this herd cannot be stated, since the number of animals observed was small, and the observations were not continued beyond the second period. The significance from the standpoint of the user of milk, individual or manufacturer, seems slight, since the composition of the total amount of milk produced at a milking was within ranges considered as normal.

The question whether the physiological disturbance studied provides a favorable environment for *Streptococcus agalactiae* was discussed in the previous report, and further observations have been made by exposing some of the animals described herein to infection with *S. agalactiae*. The tentative conclusion is that the environment provided in the abnormal quarters is more favorable for the establishment of streptococci than that provided by the normal gland.

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[3] JOHNS, C. K., and HASTINGS, E. G. CONCERNING THE USE OF INDIRECT BIOCHEMICAL TESTS FOR THE DIAGNOSIS OF CHRONIC CONTAGIOUS MASTITIS. Canad. Jour. Research 16 (D. Zool. Sci.): 6-14, illus. 1938.

SUMMARY

The observations reported in an earlier paper regarding the production of abnormal foremilk in the first lactation period by cows free from udder streptococci have been extended for some of these animals during a second lactation period. The continued study does not permit any definite statement as to the cause of the disturbance or the transmissibility of its cause.

The disturbance tends to continue from one lactation period to another in the same quarters. It also tends to decrease production and to shorten the production period. The milk apparently is rarely so changed in composition as to be of less value either as fluid milk or as a raw material for the various milk products.

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ANATOMY OF THE HYPOCOTYL AND ROOTS OF DAUCUS CAROTA[1]

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INTRODUCTION

The cultivated carrot (*Daucus carota* L.) is a biennial plant of the Umbelliferae family. The first year of its growth the hypocotyl and the upper portion of the primary root become fleshy. The stem is very short during this first year, but, during the second growing season, a flower stalk extending to a height of 2 to 4 feet is produced. In the study here reported an attempt has been made to follow the anatomical development of the root and hypocotyl from the seedling stage to edible maturity, that is, to the end of the first season.

REVIEW OF LITERATURE

The anatomy of the hypocotyl and roots of the carrot apparently has never been studied in detail. A few studies, however, have been made of various phases of the anatomy of several plants belonging to the Umbelliferae family. Gérard (*3*)[2] reported, in a general way, the transition from root to stem structure in several plants of the Umbelliferae, but he did not mention the carrot. Van Tieghem (*7*) and Van Tieghem and Douliot (*8*) described the origin of lateral roots from the primary one in several plants of this family. Apparently, however, they made no specific study of the carrot in this regard. Van Tieghem (*7*) also noted the oil ducts in several members of the Umbelliferae. Trécul (*6*) described briefly the characteristic vessels found in a number of plants of the same family. He also mentioned the secretory canals found in several plants of this family including the carrot. Courchet (*2*) reported on some of the gross anatomical characteristics of the Umbelliferae. Warning (*9*) recently reported studies on the anatomy of the vegetative organs of the parsnip. From her studies it seems that in many respects the carrot and parsnip are quite similar in certain anatomical characters but strikingly different in others.

MATERIAL AND METHODS

Plants at successive stages of development were used in this study. The plants used were mainly of the Chantenay variety and were grown in the greenhouses of the Ohio Agricultural Experiment Station. Plants 8 to 10 days old proved most satisfactory for the study of the primary body of the hypocotyl and roots. From this stage to maturity numerous other specimens were secured throughout the growth of the plants.

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[1] Received for publication April 26, 1938.
[2] Italic numbers in parentheses refer to Literature Cited, p. 564.

The specimens were killed, dehydrated, and embedded in paraffin by the butyl alcohol method. The transverse and longitudinal sections were cut 10 to 12μ in thickness, mounted serially, and stained, usually with safranine and fast green.

GROSS MORPHOLOGY OF THE SEEDLING

The initial development of the seedling is shown in the rupturing of the seed coat by the development of the hypocotyl and the radicle. The elongation of the inverted V-shaped hypocotyl brings the cotyledons out of the soil and the hypocotyl into a vertical position. In the greenhouse at 60° to 70° F. this entire process is completed in 5 to 6 days. The cotyledons are oblanceolate and radiate-veined, and the petioles are 5 to 6 mm. in length. As is the case in many seedlings, the cotyledons are undiverged at their bases and form a cotyledonary tube in which the epicotyl is located. In the carrot this tube is relatively short, extending only 30 to 40 μ above the cotyledonary node.

In the carrot seedling the upper extremity of the primary root may be located by the smaller diameter of the root in contrast to the hypocotyl above it. Furthermore, most lateral roots arise from the primary root although often a few roots may be seen developing from the lower portion of the hypocotyl of the seedling. The upper extremity of the hypocotyl is terminated by the cotyledonary node. The hypocotyl is usually 15 to 25 mm. in length; the actual length depends largely on the moisture, soil, temperature, and light conditions under which the plants are grown.

ANATOMY OF THE SEEDLING

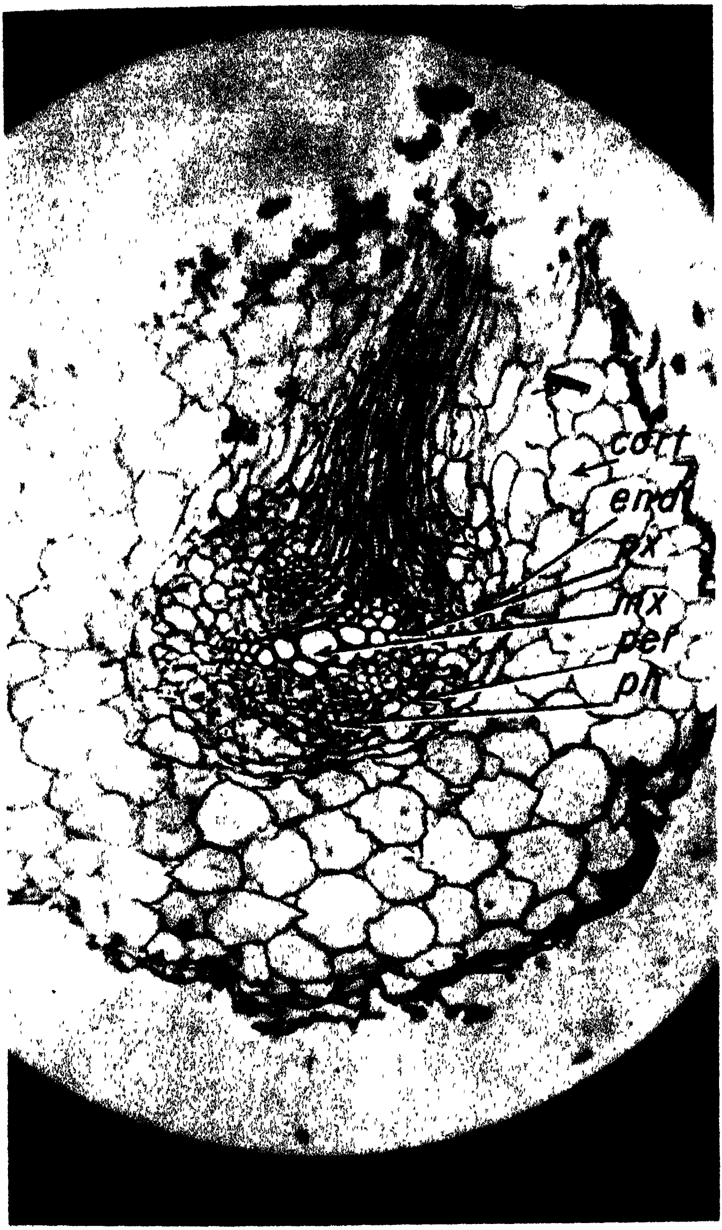
THE PRIMARY ROOT

The structure of the primary root of the carrot seedling as it appears 8 to 10 days after the seed is planted is not at all unusual. The xylem and phloem are radially arranged in the primary body of the young root (pl. 1). The xylem is diarch and is composed of four to six protoxylem elements which are abaxially arranged with respect to the six to eight metaxylem cells. No pith was found in the root. The phloem is separated from the diarch xylem by three to four layers of parenchyma, one of which will form the cambium. The primary phloem is composed of sieve tubes, companion cells, and parenchyma.

In the young primary root the pericycle is composed of one or two layers of rather irregularly shaped parenchymatous cells. This sheath of pericycle surrounds the phloem and abuts the two protoxylem points. Lateral roots originate from the pericycle, usually near a protoxylem point but not opposite it (pl. 1). The endodermal cells vary in size and shape, but as a whole they are larger than those of the pericycle. The Casparian thickenings (of the endodermis) are seen as somewhat inconspicuous dots or strips along the radial cell walls. The cortex is composed of five to seven layers of irregularly shaped parenchymatous cells. In this parenchyma the intercellular spaces are comparatively large. The epidermis is one layer thick and is the first tissue to differentiate from the promeristem, followed closely by the cortex.

THE YOUNG HYPOCOTYL

As previously stated, the hypocotyl of the carrot seedling is usually 15 to 25 mm. in length, and lateral roots may arise from the lower portion of it. The transition from root to stem structure occurs in



Transverse section of young primary root. Note diarch xylem, radial arrangement of xylem and phloem, and origin of lateral root: *cort*, Cortex; *end*, endodermis; *px*, protoxylem; *mx*, metaxylem; *per*, pericycle; *ph*, phloem. × 280.

the upper one-seventh or one-eighth of the hypocotyl (2 to 3 mm.): thus the larger part of the hypocotyl is rootlike in anatomical structure. The anatomical description already given of the young primary root is applicable to six-sevenths to seven-eights of the hypocotyl.

THE TRANSITION ZONE

Below are given the anatomical changes which occur in the transition from root to stem structure in the primary body, beginning with the upper limit of rootlike structure of the hypocotyl and continuing through the upper hypocotyl to the base of the cotyledons, where the transition is complete. A reorientation of the primary body is first evident 2 to 3 mm. from the cotyledonary node (fig. 1, *A*). Here a

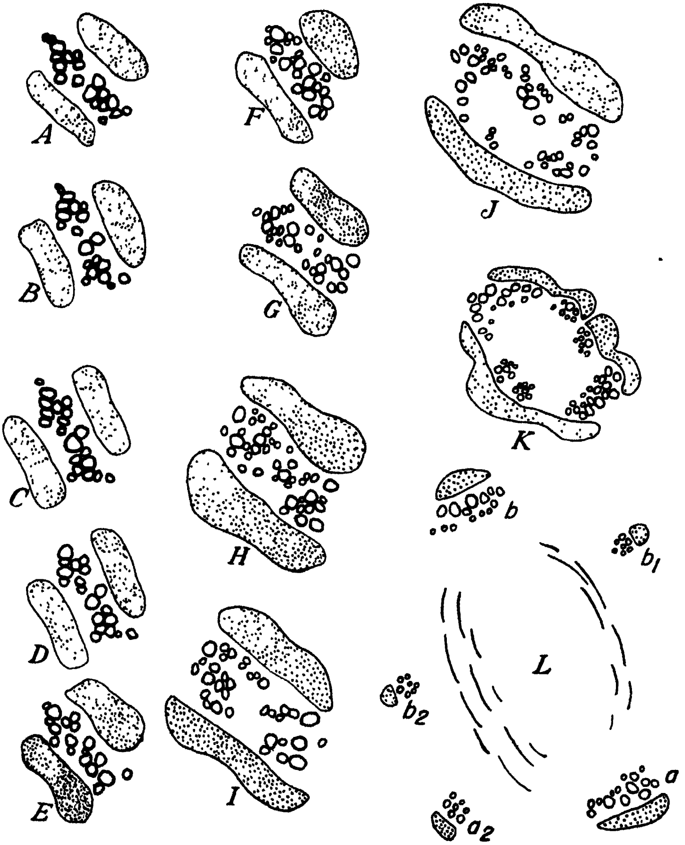


FIGURE 1.—*A–K*, Partly diagrammatic representation of the primary vascular system of the transition zone in series from the upper part of the hypocotyl into the cotyledons. *L*, *a* and *b*, midribs of the two cotyledons; a_1, a_2, and b_1, b_2, lateral bundles of the two cotyledons respectively. The phloem is stippled in all cases.

small pith is present, and the protoxylem points are not so distinct (fig. 1 *A*, *B*, *C*) as below that region. As a higher level is reached, the primary xylem elements are more and more separate from each other and are more numerous (fig. 1, *C*, *D*, *E*, *F*, *G*). The protoxylem becomes more adaxially arranged, and the metaxylem is differentiated toward the outside or to an abaxial position. The primary phloem shows little change until a position only 1 to 1½ mm. from the cotyledonary node is reached (fig. 1, *H*). The phloem then is seen to be differentiated more and more around the xylem as a higher plane is cut (fig. 1, *I*, *J*, *K*) until it almost encircles it.

About 1 mm. from the cotyledonary node the primary xylem elements form six groups (fig. 1, *J*, *K*). Here the phloem is organized around these xylem groups, and a collateral arrangement of the xylem and phloem results (fig. 1, *K*, *L*). This is usually at the cotyledonary node. The bundles are endarch as well as collateral here where they diverge into the cotyledons in most carrot seedlings, although in some cases the transition is completed in the bases of the cotyledons. The larger bundles (fig. 1, *L*, *a* and *b*) make up the midribs of the two cotyledons and the smaller bundles (fig. 1, *L*, a_1, a_2, b_1, and b_2) make up the lateral bundles of the two cotyledons respectively. For example a_1 and a_2 represent lateral bundles which diverge into the same cotyledon as does the midrib represented by *a* (fig. 1, *L*).

It may be noted that, as in salsify (*4*), dahlia (*5*), and many other plants, the primary vascular bundles of the root and hypocotyl are not continuous with those of the epicotyl. The primary bundles of the hypoctyl diverge entirely into the two cotyledons, as already described. The endarch collateral bundles of the epicotyl are differentiated soon after the primary bundles are formed in the hypocotyl. In the older plant, of course, bundles in the root, hypocotyl, and stem are continuous.

ONTOGENY OF THE HYPOCOTYL AND ROOTS

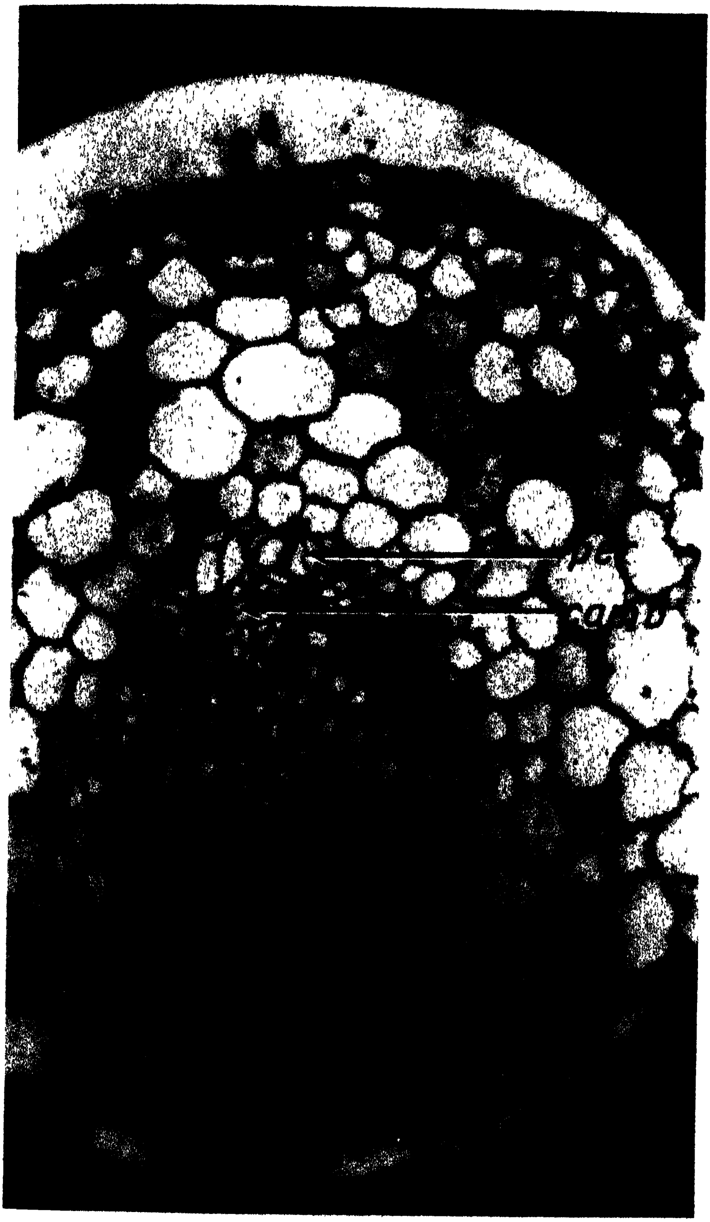
THE HYPOCOTYL AND UPPER TAPROOT

The edible portion of the carrot is largely hypocotyl and varying amounts of the upper taproot, the relative amount of taproot depending somewhat on the horticultural variety. Thus, with the exception of the few millimeters of transition zone at the upper part of the hypocotyl, the enlarged edible portion of the mature carrot is anatomically of root structure.

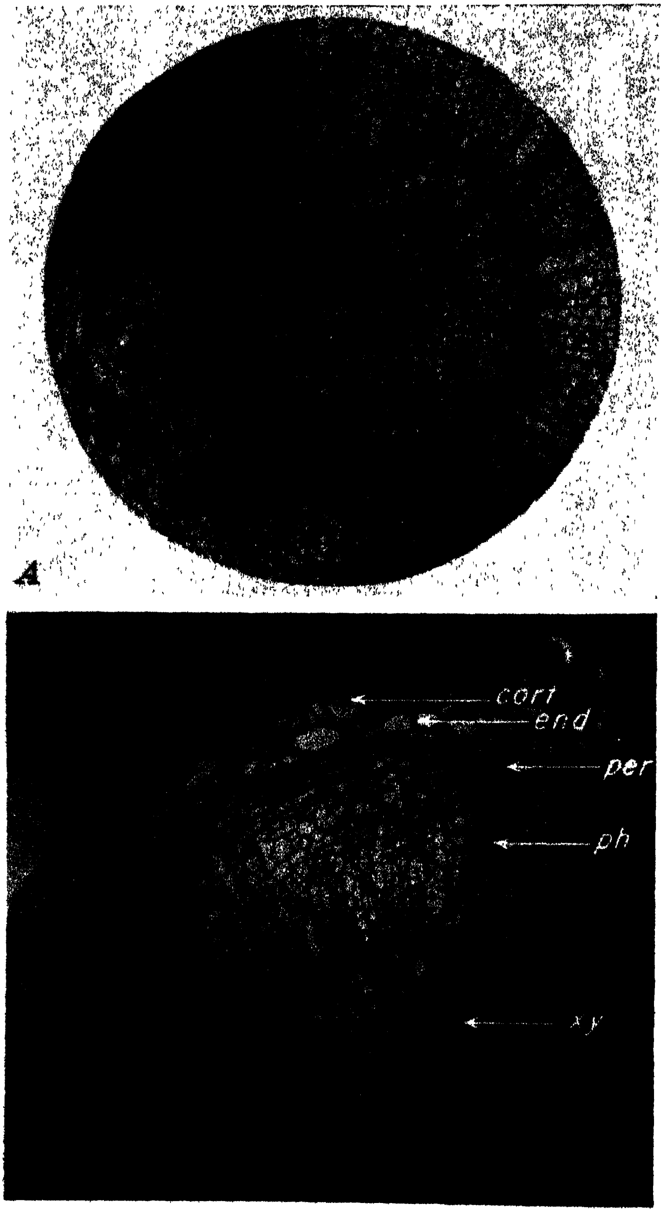
Early Secondary Thickening

The first evidence of secondary thickening is noted in the procambium or parenchyma between the primary xylem and phloem groups. These cells first elongate axially; then tangential division takes place. Thus the primary cambium originates, and secondary xylem and phloem is developed in the usual manner (pl. 2). Tracheae, tracheids, and considerable parenchyma are laid down in the xylem; meanwhile sieve tubes, companion cells, and parenchyma are developed in the phloem.

Almost simultaneous with the development of the primary cambium near the phloem poles is the division of the pericycle parenchyma opposite the protoxylem points. After only a few divisions of these



Early secondary thickening of hypocotyl. Note activity of pericycle and interfascicular cambium: *per*, Pericycle; *camb*, cambium. × 300.



Transverse section through upper hypocotyl showing growth and development of the various tissues. *A*, Central tissues at about the time the cortex is shed. × 115. *B*, Sector showing enlargement of hypocotyl just before the cortex is shed: *cort*, Cortex; *end*, endodermis; *per*, pericycle; *ph*, phloem; *xy*, xylem. × 155.

cells, an interfascicular cambium is formed from the pericycle which becomes continuous with the primary cambium between the xylem and phloem previously described (pl. 2). This interfascicular cambium does not form immediately a cylinder of secondary vascular tissue but for some time forms parenchyma only. In this way two wide parenchymatous rays are developed opposite the protoxylem points (pls. 2 and 3).

Shortly after the formation of the first secondary thickening a small amount of radial cell division takes place in the endodermis; thus this tissue remains one cell in thickness, but keeps pace with the enlargement of the stele. The cortical parenchyma thickens very little, although there is a small amount of radial cell division here during the early stages of secondary thickening.

PERIOD OF FURTHER ENLARGEMENT

The primary xylem, especially the protoxylem, becomes so distorted as secondary growth continues that it is soon hardly recognizable. However, the original relative positions of the points may easily be located since the primary medullary rays of parenchyma are always opposite them (pl. 3). During the growth in diameter of the fleshy root and the hypocotyl of the carrot, the xylem, as well as the phloem parenchyma, remains meristematic, although to a lesser degree than the pericycle. The division and enlargement of the parenchyma is especially active in about the upper one-fourth of the hypocotyl (pl. 3). In fact, the pithlike center of the upper portion of the mature edible carrot is almost entirely xylem parenchyma, although there is a small amount of pith in the transition zone, which, as previously described, extends only a few millimeters below the cotyledonary node. Both *A* and *B* of plate 3 were cut through the lower part of the transition zone.

Eventually the cambium opposite the protoxylem points lays down tracheae, tracheids, and considerable parenchyma in the xylem and a relatively small number of seive tubes, companion cells, and parenchyma in the phloem. Thus, while the hypocotyl and enlarging root are only 3 to 5 mm. in diameter, there is a continuous ring of secondary vascular tissues. Several rays of secondary xylem parenchyma are usually present, across which the interfascicular cambium is active. These rays in the secondary vascular tissue are sometimes opposite the protoxylem points, although more often they are at angles of 30° to 50° with the original protoxylem position (pl. 3). At right angles to the primary xylem the cambium is extremely active. At these two opposite positions xylem and phloem are developed with a considerable amount of parenchyma. Here the phloem extends almost to the endodermis, indicating that the pericycle is relatively inactive at these places. However, opposite the original protoxylem points and early parenchymatous rays the phloem is relatively narrow, and the pericycle is quite active.

The endodermal cells continue to divide radially as the fleshy part of the carrot develops; thus the endodermis remains one layer thick (pl. 3, *B*).

Very little cell division takes place in the cortex. Because of the pressure from the growing stele the cortical cells become flattened tangentially, and the cortex is shed when the fleshy root and hypocotyl are 3 to 5 mm. in diameter (pl. 3, *B*). Just before the cortex is shed, a dermatogen layer begins to form from the pericyclic cells just inside the endodermis (pl. 3, *B*).

THE MATURE STRUCTURE

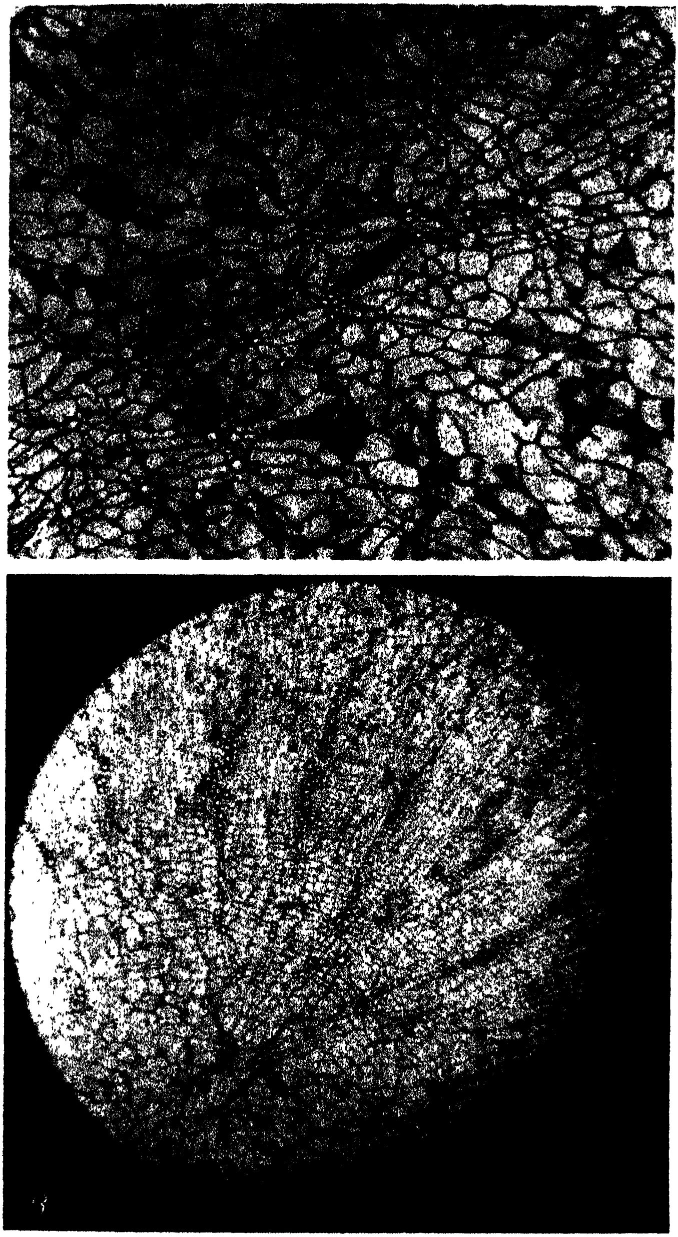
The mature enlarged or fleshy root and hypocotyl of *Daucus carota* is composed largely of parenchyma. If one examines a cross section of a mature carrot (enlarged part) with the unaided eye a pithlike center is noted. This is relatively large at the upper part of the hypocotyl and is smaller as a lower region is reached. With the exception of the few millimeters of transition zone in the upper hypocotyl, this pithlike zone consists largely of thin-walled xylem parenchyma and not true pith. At this stage the primary xylem usually cannot be identified since the protoxylem has disintegrated and the metaxylem has become scattered throughout this central area (pls. 4 and 5). This phenomenon is due mainly to the enlargement of the central parenchymatous xylem cells, although some division of these cells occurs (pl. 4, *A*). In the short transition zone there is a true pith composed of thin-walled parenchyma which is surrounded by the primary xylem in the younger plant. The primary xylem, here too, usually loses its identity in the mature structure owing to the enlargement and division of the pith and xylem parenchyma. In the lower root region, where much less enlargement takes place, there is little proliferation of the parenchyma, and the parenchymatous cells become lignified relatively early. The primary xylem remains intact here.

The parenchymatous rays in the xylem of the mature enlarged root and hypocotyl increase in size mainly through cell enlargement, although some cell division occurs (pl. 4, *B*). In the mature structure the two broad bands of parenchyma originally opposite the protoxylem points still persist. The vessels lose much of the ray arrangement noted earlier in the ontogeny because of the activity of the parenchyma surrounding them (pl. 5). The size of the xylem, or "core," in proportion to the thickness of the tissues surrounding it (separated by cambium) varies considerably with the carrot variety. A relatively small core is usually associated with a high-quality variety.

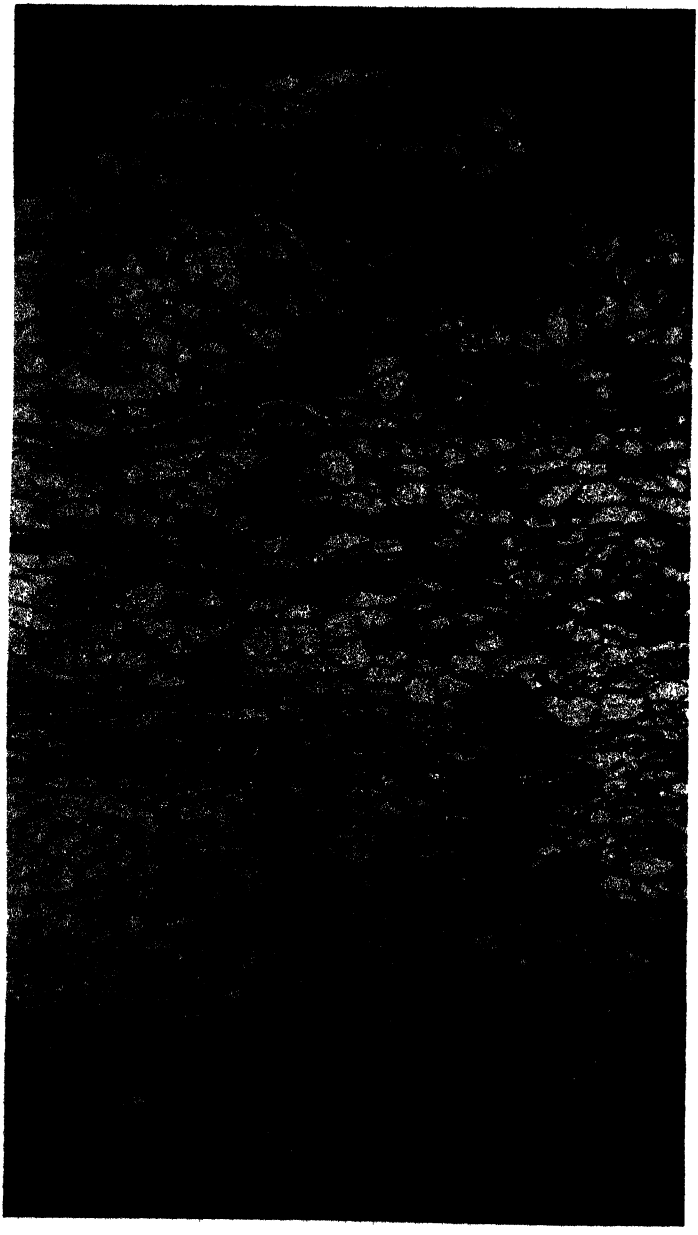
The primary cambium remains active as the edible carrot reaches maturity. The cambial region is composed of four to six rows of thin-walled, compact, rectangular cells, as seen in transverse sections.

The phloem of the mature structure consists of a relatively large amount of parenchyma, although there are also sieve tubes, companion cells, and a few fibers. There are two large groups of phloem located opposite each other and radially arranged with respect to the original primary xylem. The two groups of primary phloem which, earlier in the ontogeny, were located on the outer edge of these large secondary phloem groups, disintegrate until they cannot be located in the mature carrot. These two secondary phloem groups extend to within a few cells of the periderm of pericyclic origin. Oil ducts are also present near the periphery of the phloem (pl. 6, *A*).

The pericycle makes up about a third as much of the region outside of the cambium as the phloem. It is only a few layers in thickness opposite the two large phloem groups described in the preceding paragraph, but in other localities it is much thicker. The pericycle remains meristematic throughout the growing season; both radial and



Transverse sections through central regions of enlarged hypocotyl: *A*, Distortion of primary xylem and enlargement and division of central xylem parenchyma. × 252. *B*, The same as *A*, showing also principal xylem rays, cambium, and phloem. × 84.



Longitudinal section through hypocotyl cut at about the same stage and place as transverse section shown in plate 4, *A*. Note distortion of vessels by growth of parenchyma. × 200.

tangential division of cells takes place (pl. 6, *A*). Oil ducts are present near the periphery of the pericycle. These are quite similar in their development to those described by Trécul (*6*) as characteristic of the Umbelliferae, and also to those of the parsnip as described by Warning (*9*). The periderm, formed from pericyclic cells, is five to eight cells in width (pl. 6, *A*). The phellogen forms cork cells on the outside and phelloderm cells inside. The number of cork cells formed is much greater than the number of phelloderm cells, since the cork cells are constantly sloughed off and replaced.

LATERAL ROOTS

The lateral roots of the carrot originate in the pericycle of the primary or taproot at an angle of about 45° with the primary xylem group (pl. 1). This is also true of roots which originate in the lower hypocotyl since it is rootlike in anatomical structure. There are thus four rows of lateral roots located about 90° apart on the diarch tap root and hypocotyl. Van Tieghem (*7*) cites several plants of the Umbelliferae in which he found that lateral roots arose at a 45° angle to the primary xylem. He states that the lateral roots arise by the division of the pericyclic cells on radii between the vessels and the phloem of the primary root. He notes further that the pericycle opposite the protoxylem points has no "caractère spécial" which prevents the origin of rootlets, and that he does not know the reason for their failure to originate there.

As the lateral root originates, the endodermal cells at the tip of this root elongate and then divide as the root continues to develop; finally the endodermis is ruptured. The lateral roots develop through the cortex by mechanical pushing and also by digestion of cortical cells.

The general structure of the lateral roots is similar to that of the lower primary root. There is relatively less thin-walled parenchyma, however, and more lignified cells. The secondary lateral roots, as well as the tertiary ones, are diarch. Phloem fibers are developed early and are relatively numerous (pl. 6, *B*).

Lateral roots often arise at the periphery of the pericycle during early secondary growth of the primary or taproot and the hypocotyl (pl. 7, *A*) and also after the cortex has disappeared (pl. 7, *B*). Similar phenomena have been observed in the beet by Artschwager (*1*) and in the parsnip by Warning (*9*). In the case of the carrot this type of lateral roots seems to arise always from the pericycle near the place of emergence of the endogenous lateral roots already described. Thus while only one root, which may or may not be permanent, arises endogenously at a specific point, there is often a group of several roots opposite a root trace in the older carrot.

SUMMARY

The carrot seedling has oblanceolate, radiate-veined cotyledons, which are photosynthetic. The epicotyl is enclosed in a short cotyledonary tube. Lateral roots arise from the lower hypocotyl as well as from the primary root.

The transition zone in the seedling is located in the upper part of the hypocotyl and is relatively short. Thus, most of the hypocotyl is rootlike in anatomical structure. The primary root, as well as the lateral roots, is diarch.

From a morphological viewpoint the enlarged or edible part of the carrot is largely hypocotyl, although the upper taproot is enlarged often also, the relative amounts depending on the horticultural variety.

In the early enlargement of the edible portion of the carrot the cambium and the pericycle are quite active. As the carrot develops further the xylem and phloem parenchyma become meristematic, and there is considerable enlargement especially of the xylem parenchyma. The cortex is shed when the fleshy root and hypocotyl are 3 to 5 mm. in diameter. The cork cambium is then derived from the pericycle. The mature enlarged structure is composed largely of xylem, phloem, and pericyclic parenchyma.

Lateral roots originate in the pericycle and at an angle of about 45° with the primary xylem cylinder. Thus, there are four rows of lateral roots located 90° apart on the diarch taproot and hypocotyl. The secondary, as well as tertiary, lateral roots are diarch. Phloem fibers are developed here early and are relatively numerous.

Lateral roots also often arise at the periphery of the pericycle during early secondary growth and also after the cortex of the hypocotyl has disappeared. This type of lateral root arises near the place of emergence of the endogenous lateral root.

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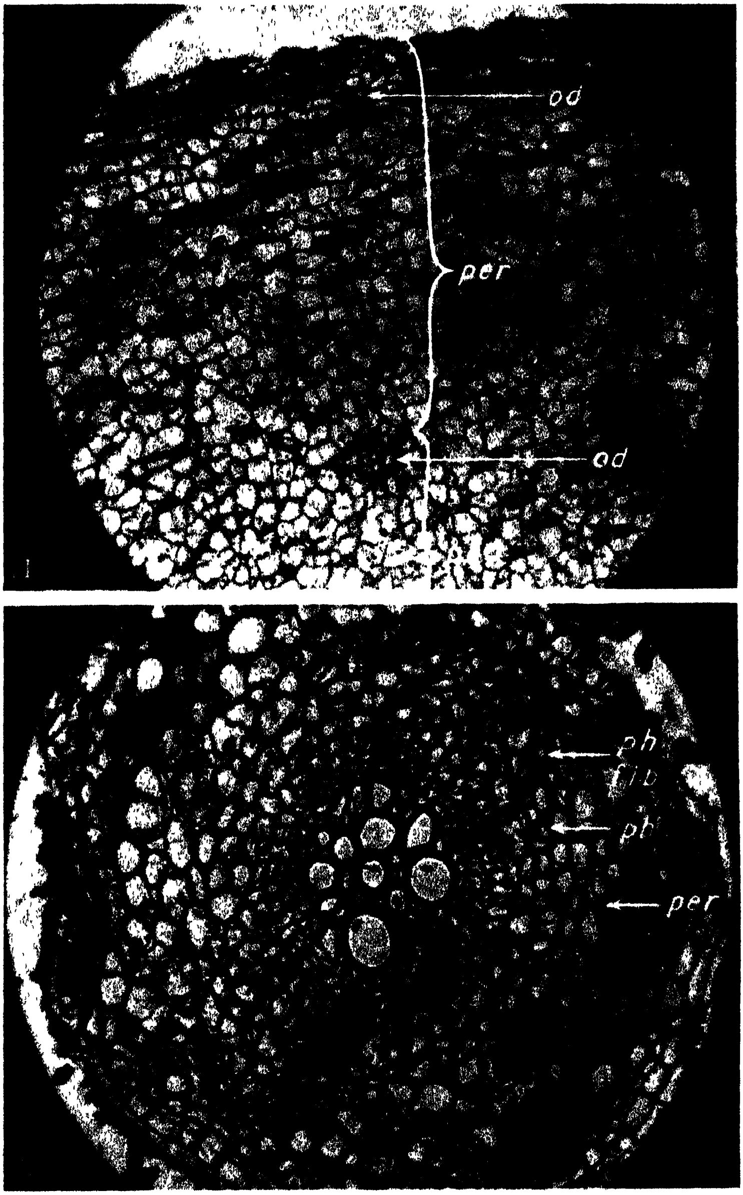
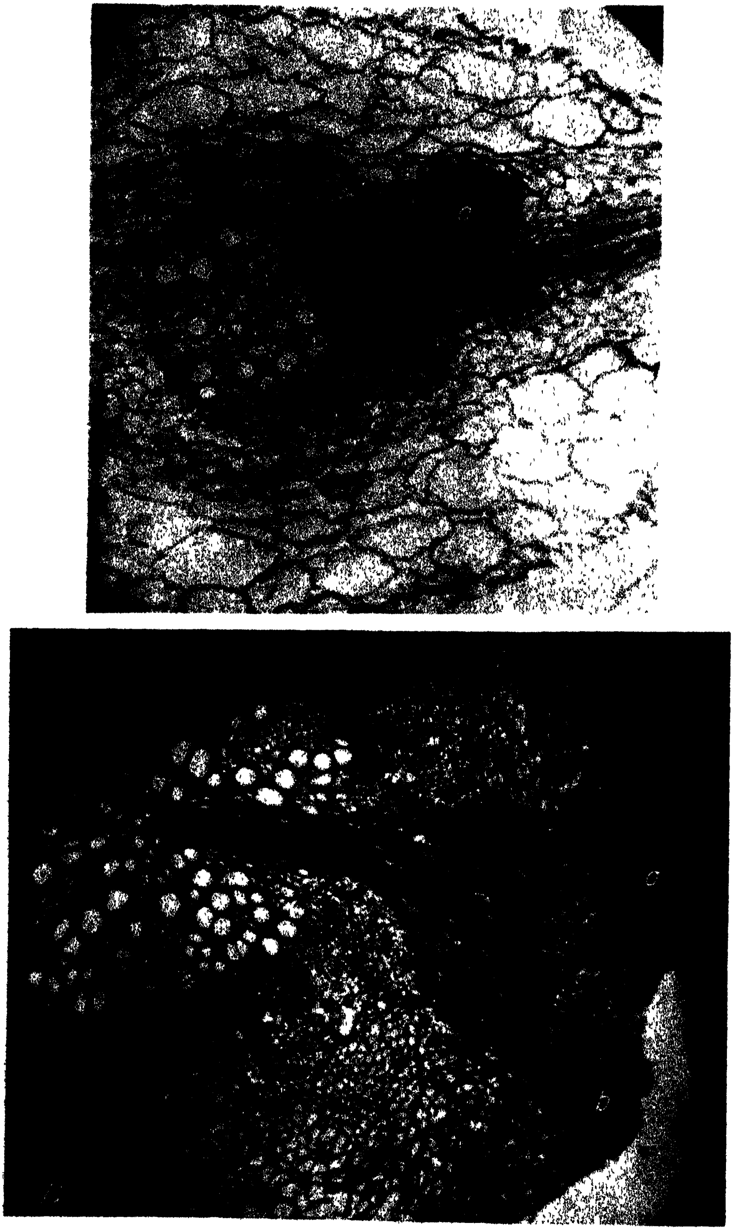


A, Transverse section through outer part of enlarged hypocotyl. Note active pericycle (*per*) and phloem (*ph*) and also the oil ducts (*od*). × 200. *B*, Cross section of a secondary root. Note numerous phloem fibers and lack of thin-walled parenchyma in both xylem and phloem: *ph*, Phloem; *ph fib*, phloem fibers; *per*, pericycle. × 200.



Origin of lateral roots during secondary growth from the primary root: *A*, Lateral root (*a*) originating from pericycle near trace formed by endogenous one, cortex still present. *B*, Origin of lateral roots (*a* and *b*) from older primary root after cortex is shed. × 103.

PRENATAL AND POSTNATAL SEX RATIO IN SHEEP [1]

By William Lewis Henning

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INTRODUCTION

Modern evidence indicates that the sex chromosome from the male gamete is the initial determiner of sex in mammals. A necessary implication of this mechanism is that sex of a zygote is determined at fertilization. Cytological studies indicate that the male gametes bearing, respectively, the X and the Y chromosome are produced in equal numbers. Unless factors other than chance interfere, the sex ratio at fertilization should accordingly be one of equality. In the absence of sex reversal, later change can come about in the ratio between the sexes only as the result of a differential mortality.

Sex ratios (percentages of males) may be ascertained at different stages in the life history of a population. In mammals, it is convenient to consider three separate stages: at conception, birth, and maturity. Sex ratios at these stages are usually spoken of as primary, secondary, and tertiary, respectively.

The true primary sex ratio might conceivably be obtained by a cytological study of a large number of fertilized eggs. The ratio would depend upon the proportion of ova fertilized by the male- and female-determining spermatozoa, respectively. However, adequate numbers of fertilized mammalian eggs for such a study, in which the cytological picture could be determined, would be difficult to obtain. It is also impracticable to make sex determinations on a sufficiently large number of embryos before external differentiation of the genitalia. The immense labor involved precludes the histological examination of the gonads of any large number of embryos as a laboratory method. Actually, therefore, primary sex ratios have not been determined, but an approach is made by a study of the percentage of males in fetal life. This method of approach, then, will give the sex ratio as early in prenatal life as the sexes can be determined macroscopically. It leaves the very early stages as a matter of speculation.

Sex ratios at birth in most mammals have been found to approach equality. In contrast to this, however, several studies of fetal sex ratios have indicated a preponderance of males before birth. In some cases this percentage has been reported as being high in early fetal stages and showing a gradual decline toward birth. Such a trend would indicate a higher proportion of males at conception than at any other time in the life cycle. Most authors have assumed that with a high primary sex ratio, differential prenatal mortality tends to

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equalize the sexes by the time of birth. The only adequate data which give no evidence of difference between the prenatal and secondary sex ratios are those of MacDowell and Lord (*17*)[3] on the sex ratio of litters in the mouse.

The tertiary sex ratio is commonly applied to animals in the wild state where there are few artificial factors causing a differential mortality from birth to maturity. The tertiary sex ratio in domestic animals has little biological meaning because of the polygamous use of breeding males and the elimination of many males before they reach maturity.

The present paper reports the results of a study made on the prenatal and postnatal sex ratio in sheep.

REVIEW OF LITERATURE

General reviews of the literature on sex ratios have been presented by Parkes (*27*) and more recently by Johansson (*13*). Several authors have reported the secondary sex ratios in sheep. Their data have been obtained from flock book records, breeders' replies, and lambing records. A summary of the available secondary sex ratios reported in sheep (table 1) gives a male percentage of 48.96±0.14 on a total of 127,587 individuals. This is slightly lower than 49.5 percent, reported by Lush (*16*). The fact that breeders' replies for some of the earlier work reported gave the ratio at castration time, and hence not strictly secondary ratios, may explain the slightly lower value for those records. McPhee (*21*) has shown for swine that herdbook data do not possess the necessary accuracy for a study of sex ratios and frequency of sex combinations.

TABLE 1.—*Sex ratios reported in sheep by various investigators*

Breed	Total amounts	Males	Authority	Source of data
	Number	*Percent*		
Leicester	8,965	44 07±0 48	Darwin (*9*)	Breeders' records—castration ratio
Cheviot and Blackface.	50,685	49 40± .22	do	Do
Cross-bred and Hampshire.	6,751	49 34± .61	Wilckens (*31*)	Breeders' replies
Several breeds	821	49 94±1.74	Carlyle and McConnell (2).	Lambing records—Wisconsin Station.
Mixed breeding	371	54 40±2.58	Popenoe (*29*)	Bell's records
Do	2,623	49 50± .98	Nichols (*23*)	Breeders' replies.
Do	17,201	49.34± 38	do	Breeders' replies—castration ratio
Southdown	5,014	51.02± .71	Nichols, (*24*)	Private records
Blackface	9,549	48 27± .51	Nichols, (*25*)	Breeders' records—castration ratio
Cheviot	10,512	48 99± .49	do	Infantile ratio.
Several breeds	13,241	49.30± .43	Johansson (*14*)	Private records.
Grade Hampshire	1,019	48.40±1 57	Chapman and Lush (*4*)	Lambing records—Iowa Station
Southdown	835	55.20±1.72	Phillips personal communication.	Lambing records—U. S. Department of Agriculture, Beltsville, Md.
Total	127,587	48.96±0.14		

Several authors have reported on the prenatal sex ratio of other mammals. The only direct evidence that the ratio at conception is not higher than that at birth is the investigation by MacDowell and

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[3] Italic numbers in parentheses refer to Literature Cited, p. 578.

Lord (*17, 18, 19*), previously mentioned. They found that the sex ratio of 68 litters of mice, which was determined by corpora lutea counts, suffered no differential prenatal mortality. Of the 523 young in these litters, 261 were male, and 262 female (49.9 percent males), a ratio indistinguishable from the normal ratio at birth.

Information on sex ratio at birth and sex incidence of fetal mortality has been used to calculate a probable figure for the ratio at conception in man. Parkes (*28*) presented such material in a graph showing the decline in the sex ratio of mortality for the determinable part of the period of gestation as nearly linear. He then extrapolated the curve to the time of conception, thus arriving at a presumable sex ratio of mortality during the first 3 months of gestation, in which sex cannot be observed. He concluded that the primary sex ratio is probably about 120 males to 100 females (54.54 percent males).

Lillie (*15*), in his work on the freemartin, found that the fetal sex ratio for dizygotic twins in cattle was 134, which is much higher than the secondary sex ratio, 106, given by Johansson (*13*) for all births. Lillie did not classify the fetuses in age groups to ascertain if there was a trend in the mortality for the different stages of development. Jewell (*12*) found that the sex ratio of 1,000 cattle fetuses was 123.21 to 100, but observed no definite relation to stage of development.[4]

Parkes (*26*) found that the percentage of males was 56.8±1.38 among 583 pig fetuses of all ages. This is higher than that of 51.14± 0.18 for pigs at birth, summarized from various authors by Lush (*16*). In addition, when these pig fetuses were classified by weight (0–100, 101–300 g, 301+g) the percentages for males were 59.1, 57.0, and 53.2, respectively. The percentage of males decreases as gestation proceeds, and Parkes concludes that this result can only be brought about by a differential mortality of males and females. Extrapolation of the foregoing data shows the primary sex ratio to be nearly 60 percent. Crew (*7*) made a similar investigation on the fetal pig and also concluded that the primary sex ratio must be at least 60 percent.

It appears that the only data recorded on sex of prenatal sheep are those of Malan and Curson (*22*), who report seven male and two female fetuses for the South African Merino.

MATERIAL, METHODS, AND RESULTS

THE SECONDARY SEX RATIO

Lambing Records of the Pennsylvania Experiment Station

The source of data for the secondary sex ratio is the lambing record of the registered flocks maintained at the Pennsylvania Agricultural Experiment Station from 1923 to 1937, inclusive. These lambing records include still births as well as lambs alive at birth and therefore are more complete than flock-book records which may be considered as the record of only the animals registered.

The mean percentage of males of 1,742 births for all breeds, including Southdown, over a period of 15 years is 50.2±1.19 (table 2). For the same period, 433 births of registered Southdowns give a percentage of males of 50.3±2.40, which does not differ significantly from that for all breeds (table 2).

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[4] At the recent meeting (Nov. 1938) of the American Society of Animal Production, Chapman, Cassida, and Côté presented a paper on "Sex ratios of fetal calves." In this paper the authors presented evidence of a high sex ratio (66 percent males) in the earliest (5 to 10 cm fetal length) stage in which sex is macroscopically distinguishable.

The data were examined to determine whether there is a significant difference in the percentage of males from year to year.

TABLE 2.—*Secondary sex ratio in sheep, 1923–37*

Year	All breeds including Southdown				Southdown			
	Males	Females	Total	Males	Males	Females	Total	Males
	Number	*Number*	*Number*	*Percent*	*Number*	*Number*	*Number*	*Percent*
1923	17	22	39	43.6±7.94	0	4	4	----------
1924	66	43	109	60.6±4.68	10	7	17	58.8±11.9
1925	46	37	83	55.4±5.46	8	6	14	57.1±13.2
1926	49	31	80	61.3±5.45	13	4	17	76.5±10.3
1927	49	46	95	51.6±5.13	16	14	30	53.3± 9.11
1928	52	65	117	44.4±4.59	8	14	22	36.4±10.2
1929	69	61	130	53.1±4.38	11	18	29	37.9± 9.01
1930	45	59	104	43.3±4.86	13	14	27	48.1± 9.62
1931	67	73	140	47.9±4.22	20	16	36	55.6± 8.28
1932	61	69	130	46.9±4.38	13	18	31	41.9± 8.86
1933	58	69	127	45.7±4.42	20	24	44	45.5± 7.51
1934	57	54	111	51.4±4.74	24	16	40	60.0± 7.75
1935	63	58	121	52.1±4.54	21	15	36	58.3± 8.22
1936	90	95	185	48.6±3.67	21	25	46	45.7± 7.34
1937	86	85	171	50.3±3.82	20	20	40	50.0± 7.91
Total	875	867	1,742	50.2±1.20	218	215	433	50.3±2.40

The extremes of variation in sex ratio are 43.3±4.86 in 1930 and 61.3±5.45 in 1926, with a mean difference of 18±4.92 percent. A chance difference of such magnitude is not unlikely when selected in this manner from a large number of differences. A χ^2 test of these 14 yearly percentages shows them not to be heterogeneous.

THE SOUTHDOWN FLOCK-BOOK RECORDS

A true tertiary sex ratio in domestic animals is difficult to obtain because of the elimination of males for economic reasons. An approach to it is made, however, by studies of herdbook records.

Flock-book data on sheep registered as association numbers 30,000 to 35,000 (16–17, American Southdown Record) were used as representing a typical cross section of registrations in this breed. Practically all of these animals are entered in the flock book before they are 1 year of age. Registrations of the lifetime progeny records of 1,536 ewes furnished the material for this study. Records were assembled in which the following information was available for each birth represented by registered progeny from these ewes: (1) Number of progeny at the birth; (2) sex of at least one registered offspring from the birth; (3) size of litter in which the dam of each birth was born, that is, whether the dam herself was dropped as a single or one of a multiple birth. The number of births for which the records yielded the desired information were 6,116 for (1), 6,065 for (2), and 6,050 for (3).

Single births constitute 55.73 percent of the lambings registered from these ewes; twin births 43.42 percent; and the remainder, less than 1 percent, triplets and quadruplets. The proportion of males is 32.44±0.80 percent for singles and 37.49±0.71 for twins (table 3).

Combined, these give 35.35 percent of males, which is the sex ratio of registration (disregarding the inconsiderable number of triplets and quadruplets).[5] The low percentage of males is accounted for by the fact that only a portion of the ram lambs are reserved and registered. For this reason, flock-book records have little value for a study of natural sex ratios and twin combinations.

TABLE 3.—*Sex ratio of registration with twin combinations as shown by the Southdown flock-book records*

Type of birth	Births	Combinations	Lambs registered	Males registered
	Number		*Number*	*Percent*
Single	1,106	1 ram	1,106	32.44±0.80
	2,303	1 ewe	2,303	
Twin	339	2 rams	678	37.49±.71
	808	1 ram, 1 ewe	1,616	
	847	2 ewes	1,694	
	257	1 ram, 1 unregistered	257	
	405	1 ewe, 1 unregistered	405	
Total	[1] 6,116		8,059	

[1] Total does not include 48 sets of triplets and 3 quadruplets.

The difference in percent of males as between single births and twins suggests that there may exist a slight preference for selecting rams that are born as one of a pair of twins.

Records secured for twin combinations in which both members of the pair were registered gave 339 ♂ ♂, 808 ♂ ♀, and 847 ♀ ♀ (table 3). The wide departure from the 1 : 2 : 1 ratio is obviously attributable to the excess of females over males registered.

Although not directly pertinent to this study, it is interesting to compare the type of birth of the offspring with that of the dam (table 4).

Of 4,309 lambings by ewes which were themselves born as singles, 58.18 percent were singles and 41.82 percent twins. In 1,741 cases in which the ewe herself was a twin, 51.23 percent of the lambings were singles and 48.77 percent twins. These data show a significant tendency for ewes themselves born as one of a pair of twins to produce more twins than do ewes born as singles. Heredity is undoubtedly a factor in producing this result, but similarity of flock conditions from one year to another may also play a part.

TABLE 4.—*Inheritance of tendency to multiple births in sheep*

Dams (number)	Type of birth	Offspring			
		Singles		Twins	
		Number	*Percent*	*Number*	*Percent*
4,309	Single	2,507	58.18±0.98	1,802	41.82±1.16
1,741	Multiple	892	51.23±1.67	849	48.77±1.71

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[5] For convenience the term "twins" is used for multiple births in general and may include an insignificant number of triplets or quadruplets unless otherwise qualified.

THE PRENATAL SEX RATIO

Fetuses were secured from ewes for which the time of the breeding that produced conception was known and hence the age of the fetus could be determined. An additional number of preserved fetuses was secured, and approximately 700 gravid uteri from freshly killed dams were examined in Chicago packing plants.

TIMED FETUSES

During the breeding season of 1934, 100 old Merino ewes were secured. They were divided into two lots and mated with two registered Shropshire rams. Records of mating were made each day by a college-trained assistant. Ewes were then sacrificed at intervals during pregnancy to secure as complete a series as possible of fetuses of definitely known ages up to and including full term. Thirty-eight such fetuses were obtained, and four ewes were permitted to lamb at normal term. The periods of gestation of these four were 147, 147, 148, and 151 days.

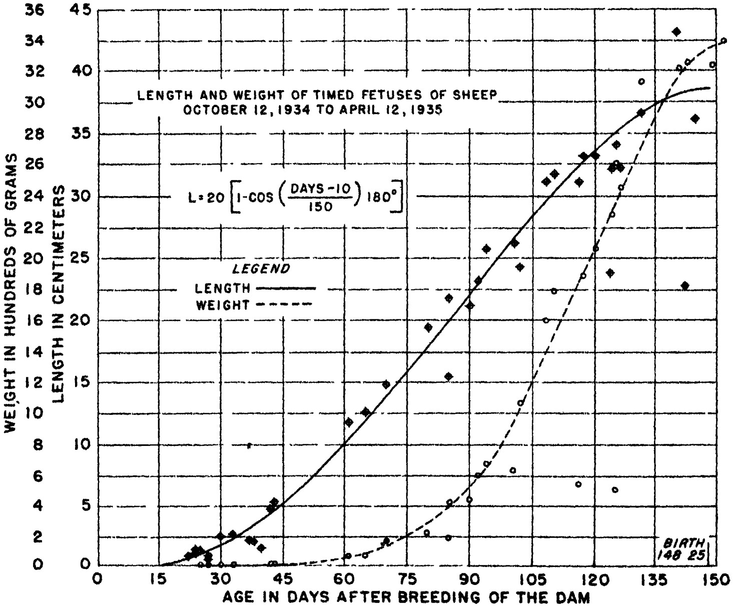

FIGURE 1.—Length and weight of fetus as related to its age in days after dam was bred

The fetus from ewe No. 47 was located in the vagina, partly resorbed but in the process of abortion. The fetuses from ewes Nos. 18, 22, and 29 were secured after the dams had died from natural causes, and this may explain the slight irregularities in the records of these fetuses. The data secured on the timed fetuses are presented in table 5. In figure 1, the two curves show the general relationship of length and weight of fetus to age in days, age being determined from date of breeding the dam. With the length of any sheep fetus known, the approximate age in days may be calculated by the following formula: [6]

$$\text{Age in days} = 10 + \tfrac{5}{6} \text{ arc cos} \left(1 - \frac{L}{20}\right)$$

(where arc cos is measured in degrees and L=length in centimeters).

The curves are very similar to those of Curson and Malan (*8*), and also those of Winters and Feuffel (*32*).

TABLE 5.—*Length and weight of sheep fetuses as related to their age and sex from work at the Pennsylvania Agricultural Experiment Station* [1]

Ewe No.	Weight of ewe	Age of fetus	Length of fetus	Weight of fetus	Sex of fetus
	Pounds	*Days*	*Centimeters*	*Grams*	
82	85 5	21	0 89	------	
94	77 0	24	1 28	------	
68	70 0	24	1 29	------	
54	91 0	24	1 38	1 4	
22 [2]	85 0	25	1. 14	1 5	
86	81 0	27	.56	------	
65	88 0	27	70	------	
58	72 0	30	2 39	5 6	
11	86.0	33	2 68	1 6	
77	76 0	37	2 09	------	
8	98 0	38	1 97	------	
47 [2]	74 0	40	1 35	------	
67	91 0	42	4 56	7 0	
85	100 0	43	5 12	8 6	Male.
51	82 0	61	11 60	56 9	Do
18 [2]	82 0	65	12 50	60 4	Do
36	73 0	70	14 70	155 9	Female.
72	81 0	80	19 30	210 0	Do
97	97 0	85	15 30	170 1	Do
88	81 0	85	21 50	411 0	Male
33	80 0	90	21.00	429 3	Do.
43	77 0	92	23 00	595 3	Female
25	67 0	94	25 50	652 0	Male.
66	74 0	101	26 00	620.0	Female.
75	68 0	102	24 00	1,047 2	Male
21	82 0	108	31 00	1,587.6	Do.
28	93.0	110	31 50	1,673 8	Do.
29 [2]	92 0	116	31 00	539.0	Do.
10	91 0	117	33 00	1,872 7	Do.
64	92 0	120	33.00	2,040 6	Female.
16	79 0	124	32 00	2,270 0	Do.
31	83 5	125	34 00	2,598 2	Male.
39	72 0	125	23 50	482 0	Female
20	92 0	126	32 00	2,440 1	Male
12	81 0	131	36 50	3,120 9	Female
95	79 0	140	43 00	3,206 3	Do
81	85 5	142	22 50	3,234 6	Male
92	90 0	144	36 00	4,876 2	Do.
6 [3]	113 0	147	------	4,082 4	Do
7 [3]	106 5	151	------	3,401 9	Female
9 [3]	95 0	148	------	3,220 6	Male
91 [3]	98 0	147	------	3,628 7	Do

[1] Data collected between Oct. 12, 1934, and Apr. 12, 1935.

[2] Ewes died or otherwise excluded.

[3] Data at parturition.

PACKING-HOUSE MATERIAL

Arrangements were made through the courtesy of Armour & Co. and Wilson & Co. to obtain sheep fetuses at their packing plants in Chicago. At first the entire uteri were placed in preservative fluid and shipped to the Department of Zoology, Pennsylvania State College, where they were measured and weighed, and the sex was recorded. Later, in order to secure larger numbers and more complete data, the work was done directly at the packing plants, the fetuses being obtained directly on the killing floors and records taken on the day of slaughter or the day following. Length was taken as the crown-rump measurement in a straight line from the cephalic curvature to caudal extremity (*1*) in all cases, with the exception of the very smallest specimens, in which the neck-rump length was secured.

All uteri containing either dead or live fetuses were used in this study. The uteri were removed by severing the vagina posterior to the cervix. At the time of collection of this material (March 3–14, 1936), many pregnant fat ewe lambs came to the market.

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[6] Credit is gratefully given to Prof. H. N. Benkert, Department of Civil Engineering, and Dr. Evan Johnson, Jr., Department of Mathematics, Pennsylvania State College, for assistance with the formula and growth curves.

Records of sex and length of fetus, together with the position and number of corpora lutea, are used in the study of the fresh material. Corpora lutea counts were not available in the preserved material.

The percentage of males for the preserved fetuses of 1933 was 43.9±7.75; for 1934 it was 41.9±5.74, and for 1935 it was 52.2±3.96. These year-to-year differences, however, are not statistically significant.

The 25 timed fetuses of 1935 classified for sex gave a percentage of males of 60.0±9.80. The percentage of males was 51.7±1.93 for 673 fetuses examined from fresh packing-house material in 1936. When all the fetuses are grouped together, there is a total of 972 (495 males, 477 females), giving a percentage of males of 50.9±1.60 (table 6). This is essentially the 1:1 ratio which would be expected if the male- and female-producing gametes are themselves produced in equal numbers, if chance alone is operating in fertilization, and if there is no sex reversal or differential mortality during intra-uterine life.

The mean percentage of males of 1,742 births for all breeds over a period of 15 years at the Pennsylvania Station is 50.2±1.20. This secondary sex ratio is essentially the same as the above prenatal sex ratio of 50.9±.60.

The fetuses were grouped in classes according to length, 5-cm differences being used as a basis of classification (table 7). These differences in length naturally correspond roughly with differences in age (fig. 1). No consistent trend in the percentage of males is apparent, and the χ^2 test shows that the variation is not significant. The same is true when they are grouped in 10- and 20-cm classes. The first group in the 5-cm classification, with a sex ratio of 80.0±6.76, is significantly different from the others, but the determination of the sex on some of these very early embryos is questionable. There is no indication, however, of differential mortality of the sexes after the 5-cm stage.

Table 6.—*Summary of data on prenatal sex ratio in sheep*

Year	Fetuses used	Singles		Twins			Triplets	Total fetuses	Males
		Male	Female	Male-male	Male-female	Female-female			
		Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
1933	Preserved material	16	19	0	2	1	0	41	43.9±7.75
1934	do	25	31	1	4	4	0	74	41.9±5.74
1935	do	81	70	0	2	2	0	159	52.2±3.96
1935	Timed fetuses	15	10	0	0	0	0	25	60.0±9.80
1936	Fresh material	254	237	25	41	22	[1] 2	673	51.7±1.93
	Total	391	367	26	49	29	2	972	50.9±1.60

[1] In 1 litter, 2 males, 1 female; in the other, 1 male, 2 females.

The prenatal sex ratio has been considered in relation to the ovary from which the ova originated (table 8). There were 232 fetuses attributed to single ovulations from the right ovary as indicated by the location of the corpora lutea. The percentage of males for these was 53.0±3.27. A total of 185 fetuses originating from ova shed singly by the left ovary gave a percentage of males of 49.2±3.67. The two percentages are not significantly different. Similarly, there was no significant difference in the percentage of males of fetuses attributable to either the right or the left ovary when there were two corpora in one and none in the other, or when there was one corpus luteum in each. The number of cases in which there were two corpora in one ovary and one in the other was so small that conclusions on this distribution are unwarranted. The percentage of males, however, for these few was 50.00.

TABLE 7.—*Prenatal sex ratio according to length of fetus*

Length of fetus (centimeters)	Males	Females	Total fetuses	Males	Length of fetus (centimeters)	Males	Females	Total fetuses	Males
	Number	*Number*	*Number*	*Percent*		*Number*	*Number*	*Number*	*Percent*
Under 5.0	28	7	35	80.0±6.76	30.0-34.9	19	18	37	51.4±8.22
5.0-9.9	81	80	161	50.3±3.94	35.0-39.9	18	7	25	72.0±8.98
10.0-14.9	133	164	297	44.8±2.89	40.0-44.9	10	9	19	52.6±11.40
15.0-19.9	115	90	205	56.1±3.46	45.0 and over	3	1	4	75.0±21.70
20.0-24.9	56	65	121	46.3±4.53					
25.0-29.9	32	36	68	47.1±6.05	Total	495	477	972	50.9±1.60

In general, no significant differences in sex ratio could be attributed to the total number of ova ovulated or to the ovary from which the ova originated. The percentage of males was 51.3±2.45 when one corpus luteum was present, 53.92±3.39 when two were present, and 50.00±14.42 when three were present. When all of the ova came from the right ovary, the percentage was 54.3±2.82; when from the left ovary, it was 49.8±3.32; and when from both ovaries at the same time, it was 50.9±4.86.

TABLE 8.—*Sex ratio of living sheep fetuses produced by right and left ovaries*

Corpora lutea in—		Fetuses appearing as—							Total fetuses	Males
		Singles		Twins			Triplets			
Right ovary	Left ovary	Male	Female	Male-male	Male-female	Female-female	Male-male-female	Male-female-female		
Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
1	0	121	109	2					232	53.0±3.27
0	1	91	94						185	49.2±3.67
2	0	10	7	22	30	12			81	58.0±5.49
1	1	16	14	16	32	16			94	51.1±5.16
0	2	7	3	8	14	10			42	52.4±7.71
2	1				2				2	50.0
1	2			2		2	3	3	10	50.0
All fetuses									646	52.2±1.96
1 corpus luteum present									417	51.3±2.45
2 corpora lutea present									217	53.9±3.39
3 corpora lutea present									12	50.0±14.42
Corpora lutea in right ovary only									313	54.3±2.82
Corpora lutea in left ovary only									227	49.8±3.32
Corpora lutea in the right and the left ovary									106	50.9±4.86

In the ovaries studied, a total of 413 corpora lutea (58.58±1.85 percent) were found in the right ovary and 292 in the left ovary. In this respect the sheep resembles other domesticated animals that have been investigated. Parkes (*26*) and Crew (*7*) found an excess of ovulations occurring in the right ovary in the pig; C. F. Clark (*5*), Casida and Venzke (*3*), and Reece and Turner (*30*) found this true in cattle; and since the present study was completed McKenzie and Terrill (*20*) have reported 56.23 percent of the ovulations in the right ovary in the ewe.

An estimate was made of the incidence of fetal mortality, based on the discrepancy between the number of corpora lutea present and the number of live fetuses in the uteri (table 9). Of necessity only those cases were studied in which there was persistent evidence of a pregnancy, that is, live or dead fetuses or remnants of fetal membranes. Consequently, cases of absence of fertilization or early embryonic death with complete resorption could not be included. In cases of multiple ovulations in which there were discrepancies between the number of corpora lutea and total number of fetuses, it is not known whether there had been additional fetuses resorbed early or the additional ova had not been fertilized. In general, therefore, the estimate of mortality (embryonic and fetal death, and failure in fertilization of eggs) must be considered as a minimum, especially in the cases of single ovulations.

Although 772 corpora lutea were observed in the ovaries associated with pregnant uteri, 16.04±1.32 percent of these were not accounted for by the presence of live fetuses. The percentage of dead or missing fetuses was 8.15±1.28 with one corpus luteum present, 26.17±2.55 with two corpora lutea, and 42.86±10.80 percent with three.

TABLE 9.—*Mortality of sheep fetuses in relation to number of corpora lutea*

Corpora lutea (number)	Fetuses found in uteri	Live	Dead or missing	Total live, dead, or missing	Dead or missing
		Number	*Number*	*Number*	*Percent*
1	1 live	415			
1	1 dead		37		
1	2 live	2			
Summary		417	37	454	8.15±1.28
2	2 live	160			
2	1 live, 1 dead or missing	60	60		
2	2 dead or missing		18		
Summary		220	78	298	26.17±2.55
3	3 live	6			
3	2 live, 1 dead or missing	6	3		
3	3 dead or missing		6		
Summary		12	9	21	42.86±10.80
Total		649	124	773	16.04±1.32

This result indicates a decidedly significant increase in the mortality of the fetuses (or possibly failure of fertilization) with increase in number of ova shed, as indicated by the number of corpora lutea. This conclusion agrees substantially with that of Hammond (*10*), who found in his study of fetal atrophy in sheep that it increased rapidly with the number of fetuses in the uterus, and that in most cases death of the fetus occurred during the early stages of development. Winters and Feuffel (*32*) state that nearly 8 percent of the eggs ovulated did not develop.

If there is a differential mortality with respect to sex during the intra-uterine period it should be reflected as a difference in sex ratios between dead and living fetuses. A summary of the sex ratio in relation to mortality is presented in table 10. The data are analyzed with reference to number of corpora lutea and number of live or dead fetuses. While the actual ratios obtained differ considerably, in no cases are their differences significant. There is, therefore, no evidence in this study for a differential intra-uterine mortality with respect to sex. This is further substantiated if only those cases are taken in which the number of corpora lutea corresponds exactly with the number of live fetuses found, and which, if the evidence is accepted, indicate no previous prenatal mortality whatever. The ratio for 575 such fetuses is 51.5±2.08. These data are similar to those of MacDowell and Lord (*17*, *18*) except that all of their sex records were at birth while these are at various stages and consequently do not exclude the possibility that there might have been some later mortality if gestation had not been terminated.

TABLE 10.—*Sex ratio in relation to sheep-fetus mortality*

Corpora lutea (number)	Fetuses found in uteri	Fetuses	Males	
		Number	*Number*	*Percent*
1	1 live	415	212	51.1±2.45
1	1 dead	16	7	43.8±12.40
2	2 live	160	84	52.5±3.95
2	1 live	57	33	57.9±6.54
2	1 or 2 dead	6	4	66.7±19.20
Live fetuses where—				
Number of corpora lutea equal number of live fetuses		575	296	51.5±2.08
Number of corpora lutea is greater than number of live fetuses.		57	33	57.9±6.54
Dead fetuses, sex determinable		22	11	50.0±10.70

MULTIPLE BIRTHS - FREQUENCY OF SEX COMBINATIONS

A study of the sex combinations in twins may be used to estimate the frequency of monozygotic twinning. In material in which no monozygotic twinning occurs and in which the sex ratio is equal, the ratios of the three possible sex combinations would be expected to approximate 1♂♂ : 2♂♀ : 1♀♀, within the limits of error of random sampling and with the provision that these ratios are not materially changed by selective prenatal mortality. Monogyzotic twinning should raise the proportion of like-sexed twins. Only limited data are available on the frequency of the different sex combinations in multiple births in sheep. Both R. T. Clark (*6*) and Johansson (*14*), who have investigated the problem, conclude that monozygotic twinning is at most a comparatively rare phenomenon in sheep.

Totals of lambing records at the Pennsylvania Station over a period of 15 years, excluding the Southdowns, gave 112♂♂ : 214♂♀ :

95♀♀, twin combinations (table 11). For the same period the Southdowns yielded sets of twins in the following ratios: 23♂♂ : 60♂♀ : 39♀♀. The χ^2 test shows the variation from the expected to be insignificant in both cases and also for the totals. The combinations of twins for all prenatal records are 26♂♂ : 49♂♀ : and 29 ♀♀; again not a significant departure from the 1:2:1 ratio.

TABLE 11.—*Twin combinations in sheep*

[From lambing records at the Pennsylvania Agricultural Experiment Station]

Item	Male-male	Male-female	Female-female
	Number	*Number*	*Number*
All breeds (excluding Southdown)	112	214	95
Southdown only	23	60	39
Total lambing records	135	274	134
Total prenatal records	26	49	29
Total all-twin records	161	323	163
	Percent	*Percent*	*Percent*
Ratio all-twin records	24.9	49.9	25.2

Grouping all the data together gives sex-combination ratios of 161♂♂ : 323♂♀ : 163 ♀♀. This total is essentially a 1 : 2 : 1 ratio, and accordingly there is no evidence from the twin combinations that there is any differential mortality of the sexes either during intrauterine life or at the time of birth.

The absence of an excess of like-sexed twins indicates that, at most, monozygotic twinning in sheep is rare. The one case of twins with only one corpus luteum present (table 9) which has already been described Henning (*11*) makes it seem highly probable, however, that monozygotic twinning may occur.

DISCUSSION

The normal secondary sex ratio (proportion of males at birth) in mammals appears in most cases for which adequate data are available to be remarkably close to 50 percent. In those species for which it is best established it varies less than 2 percent from equality. (See Lush (*16, p. 315*). The mule is not a species, and the ratio for the cat is based on embryos.) This would appear to be the natural consequence of a system of alternative sex determination based on equal numbers of unlike male gametes, providing fertilization is a random matter and differential mortality of the sexes from fertilization to parturition is slight. Under such an interpretation it might be assumed that the deviation from equality at any time during fetal life would be less than at birth. If the deviation were greater in one direction at any particular stage, because of a higher mortality of one sex, it would have to be compensated for by an increased mortality of the other sex at another stage to result in the approximate equality at birth. This would presuppose a rather nice balance of the mortality rates at different periods. The simpler expectation would be a steadily increasing deviation, except for random divergences, from a start at equality to the particular ratio characteristic for the species at birth.

Critical data on mammalian prenatal sex ratios, based on actual determinations of the sex of large numbers of fetuses of known age, are relatively few. This has been supplemented, in some instances, by records of prenatal mortality, which can be used to estimate indirectly the prenatal sex ratio. For the most part, particularly in man, the pig, and the cow, these records indicate a situation quite different from the simple straight-line divergence from initial equality suggested above. In general, in these species there appears to be a considerable excess of males during intra-uterine life and both Crew (*7*) and Parkes (*26*) have argued that this predicates an even higher primary male sex ratio. Parkes has stated this argument clearly for the pig. He concludes (*26, p. 298*):

> * * * the percentage of males decreases as gestation proceeds, a result which can only be brought about by a differential mortality of males and females. It would appear that the percentage at conception must be very near 60 per cent or 150 males per 100 females.

The initial ratio for man has been assumed to be about the same.

The careful observations of MacDowell and Lord (*17, 19*) on the mouse give results quite contrary to the conclusions drawn from previous data. These authors showed that when there was no embryonic mortality, as indicated by a correspondence between the number of corpora lutea of pregnancy and the number of young born, a 1 : 1 sex ratio resulted (*17*). Furthermore, when prenatal mortality has occurred, as shown by a discrepancy between the number of corpora lutea and the number of young in the litter, the amount of such mortality is not correlated with the sex ratio (*19*). From the first observation it is concluded that in mice "the primary sex ratio is * * * almost exactly 1 : 1," and from the second "that during gestation in mice there is no continuous selective elimination of one sex or the other before birth."

The conclusions drawn from the data on sheep, presented in this paper, appear to agree more closely with those for the mouse. It is true that the sex ratio for embryos of the smallest class (under 5 cm) is high and significantly so, but there is reason to doubt the accuracy of some of the determinations in this class. From these smallest embryos to birth the deviations are random and insignificant; certainly they show no definite trend.

How may these discordant results and conclusions for different species be explained? There are several possible suggestions:

(1) That there are species differences with respect to the primary sex ratio.

(2) That the primary sex ratio is essentially one of equality, but that in some species a high mortality of females in the early stages, before sex is ordinarily distinguishable, is followed by a later compensating mortality of males. In assuming that the trend in the later stages of pregnancy may be projected to estimate the primary sex ratio, little consideration has been given to the factors that might be causing a differential mortality during this earlier period. In the sheep, for example, distinguishable external differentiation of sex does not occur until about 45 days of pregnancy, which is about one-third of the whole period.

(3) That the data are in most cases too few or not sufficiently critical to permit definite and final conclusions.

The last suggestion emphasizes the need of more critical data, not only on the sheep, but on as many mammalian species as possible.

SUMMARY

An examination of the progeny records of the ewes among 5,000 sheep registered in the American Southdown Record gave a total of 6,116 registered Southdown births—3,409 singles, 2,656 twins, 48 triplets, and 3 quadruplets.

The frequencies of sex combinations in 1,994 complete twin-progeny records (that is, with both twins registered) gave 339 ♂ ♂ : 808 ♂ ♀ : and 847 ♀ ♀.

Of the animals registered as singles or twins in the American Southdown Record, 35.35 percent are males. This deficiency of males results from the commercial practice of reserving more females than males for breeding purposes.

A summary of 1,742 lambing records of all breeds kept at the Pennsylvania Agricultural Experiment Station gave 875 males and 867 females, with a sex ratio of 50.20 ± 1.20. Another summary of 433 lambing records of the Pennsylvania Station on registered Southdowns gave 218 males and 215 females, with a sex ratio of 50.30 ± 2.40.

The secondary sex ratio from these combined records is 50.20 ± 1.20.

A total of 972 prenatal lambs (495 males and 477 females) gave a sex ratio of 50.9 ± 1.60.

Prenatal growth curves are presented for length and weight.

A formula is given for estimating the fetal age of sheep from length in centimeters.

Mortality of sheep fetuses increases directly as the number of corpora lutea increases from 1 to 3.

There is no statistically significant difference between the sex ratios of the live and dead fetuses, or of fetuses deriving from ova from the right or left ovary, or both.

There is no evidence of a significantly higher mortality of either sex during the intra-uterine period.

Ovulation in the ewe is appreciably more frequent in the right ovary (58.58 ± 1.85 percent) than in the left.

The prenatal sex ratio found in sheep is essentially one of equality (50.9 ± 1.60).

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SCHIZONOTUS SIEBOLDI, AN IMPORTANT PARASITE OF THE IMPORTED WILLOW LEAF BEETLE (PLAGIODERA VERSICOLORA)[1]

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INTRODUCTION

Schizonotus sieboldi (Ratz.) is a chalcidoid parasite of the imported willow leaf beetle (*Plagiodera versicolora* (Laich.)) and some other closely allied chrysomelids. *Plagiodera* was first recorded in the United States by Schaeffer (*9*)[3] in 1915, but it was undoubtedly well established at that time, for in 1916 Weiss (*13*) noted its presence in three localities in New Jersey. Since then it has become a serious pest of willows in the Northeastern States. Collections made in 1932 in the vicinity of Boston, Mass., included pupae heavily parasitized by *S. sieboldi*. A study has therefore been undertaken of the biology and effectiveness of this parasite.

REVIEW OF LITERATURE

Schizonotus sieboldi has been placed in the chalcidoid family Cleonymidae by Ashmead (*1*), but Ferrière and Faure (*3*) believe it should be considered a pteromalid. Apparently very little has been written regarding the biology of any of the species of Cleonymidae. Parker has not included a representative of this family in his work on chalcidoid larvae (*7*).

Although *Schizonotus sieboldi* has frequently been noted as a parasite of *Plagiodera* and allied Chrysomelidae, about the only information recorded on its biology is that given by Cushman (*2*) in 1917. He reared it on *Chrysomela interrupta* F. and briefly noted oviposition, development, and extent of parasitization, figuring the parasite larva and pupa in situ beneath a host pupa. In 1932 Jones (*4*) recorded the numbers of *Plagiodera* pupae parasitized in small collections made at Dracut and Milton, Mass.

Brief notes on another species of *Schizonotus*, *paillotti* Ferr. and Faure, have been made by the describers (*3*). This species is a hyperparasite of *Apanteles glomeratus* L. It feeds gregariously and overwinters as a larva.

DISTRIBUTION AND HOST RELATIONSHIPS

Schizonotus sieboldi is widely distributed in the United States and Europe. It was originally described from Germany, and has been

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[1] Received for publication July 18, 1938. This study was conducted under the direction of C. W. Collins at Melrose Highlands, Mass., in 1934, and under the direction of R. C. Brown at Melrose Highlands, Mass., in 1935, and New Haven, Conn., in 1936.

[2] The writer is indebted to R. T. Webber and D. E. Parker for observations made in 1934, to H. A. Bess for making many of the pupal collections, and to Mary F. Benson for drawing the adult.

[3] Italic numbers in parentheses refer to Literature Cited, p. 592.

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recorded from France and Italy. The Division of Insect Identification, Bureau of Entomology and Plant Quarantine, records it in this country from Virginia in 1881, Florida in 1903, Minnesota in 1914, Pennsylvania in 1916, and Massachusetts in 1932, and has suggested that, since it was a parasite of native species of chrysomelids before *Plagiodera* became established in the United States, it may be a circumpolar species.

Host records, also compiled in the Division of Insect Identification, list *Schizonotus* as a parasite of *Plagiodera versicolora*, *Chrysomela interrupta*, and *C. scripta* (F.) in the United States. In the European literature it is also recorded as a parasite of *C. populi* L. by Ratzeburg (*8*), and Scheidter (*10*) reared it from *C. vigintipunctata* Scop. During the course of this study it was reared as a parasite of *Plagiodera* from every point where host material was collected, from Washington, D. C., to Bar Harbor, Maine, and west to Little Falls, N. Y. No special attempt was made, however, to get the limits of *Plagiodera* distribution.

ECONOMIC IMPORTANCE

Plagiodera versicolora passes the winter as an adult beetle under loose pieces of bark or in crevices in the tree. In the vicinity of Boston, Mass., the adults become active about May 1, and they continue to emerge from hibernation during May. The first eggs are deposited about May 15, and the first pupae are formed about June 10. There are three generations and a partial fourth during the summer. Practically all the beetles go into hibernation during August. As the various generations overlap, *Plagiodera* in all stages of development may be found in the field from June until September, although at certain times one stage may predominate.

Schizonotus is a parasite of *Plagiodera* pupae. To check its importance as a control factor, collections of pupae were made weekly during 1934 and 1935 at three points near Boston, and in 1936 one collection was made at each of these points and several collections were made in the vicinity of New Haven, Conn. Care was taken to collect only fresh pupae, i. e., pupae that had not begun to darken. In general, such pupae were not over 2 or 3 days old. If they had been collected indiscriminately, a disproportionate number would have produced parasites, for parasitized pupae may be in the field 12 or 13 days, whereas adult beetles issue from unparasitized pupae in 3½ to 4 days. An attempt was made to have each collection include 100 pupae, and at least 2 hours was spent in searching whenever the full number was not obtained. The pupae were isolated immediately upon receipt at the laboratory, and were held for emergence of adults and parasites. It would have been desirable to make all the collections on infested willow trees, but this proved very difficult, since a comparatively small number of leaves could be examined from the ground and as the season progressed many of the leaves were so dry and brown that there was little feeding on them. Consequently the pupae were collected from leaves of underbrush upon which full-grown larvae had fallen and pupated.

Tables 1 and 2 show the extent of parasitization for the various collections.

TABLE 1.—*Parasitization of Plagiodera versicolora pupae by Schizonotus sieboldi in 1934, 1935, and 1936*

Place	Date of collection	Pupae collected	Pupae dead from unknown cause	*Plagiodera* adults obtained	Pupae parasitized by *Schizonotus*	
	1934	*Number*	*Number*	*Number*	*Number*	*Percent*
Milton, Mass	July 12	100	6	12	82	82
	July 19	100	11	18	71	71
	July 25	100	5	28	67	67
	Aug. 2	28	6	5	17	60
	Aug 9	58	5	0	53	91
	1935					
	June 21	100	12	59	29	29
	June 28	100	13	44	43	43
	July 5	24	3	19	2	8
	July 11	100	12	33	55	55
	July 19	100	8	39	53	53
	July 25	100	8	15	77	77
	Aug 2	100	6	18	76	76
	Aug. 8	100	1	18	81	81
	Aug 15	56	8	12	36	64
	1936					
	July 31	100	7	25	68	68
	1934					
Belmont, Mass	July 17	100	4	51	45	45
	July 24	100	5	30	65	65
	July 31	100	24	9	67	67
	Aug 7	11	2	1	8	72
	1935					
	June 17	100	9	61	30	30
	June 24	100	22	28	50	50
	July 2	100	5	48	47	47
	July 9	100	7	73	20	20
	July 17	100	4	26	70	70
	July 23	98	12	12	74	75
	July 30	100	9	14	77	77
	Aug 6	100	10	18	72	72
	Aug. 12	100	13	31	56	56
	Aug. 19	100	12	14	74	74
	1936					
	July 31	100	11	8	81	81
	1934					
Saugus Mass.	July 13	100	2	32	66	66
	July 20	100	13	17	70	70
	July 27	100	7	63	30	30
	Aug. 3	100	10	32	58	58
	Aug 10	20	2	9	9	45
	1935					
	June 20	96	22	34	40	42
	June 26	100	15	29	56	36
	July 3	97	17	38	42	43
	July 10	100	16	69	15	15
	July 17	100	10	17	73	73
	July 23	100	8	23	69	69
	Aug 1	100	5	42	53	53
	Aug. 7	100	7	42	51	51
	Aug. 13	100	3	38	59	59
	Aug. 19	76	9	35	32	42
	1936					
	July 31	100	19	14	67	67
West Haven, Conn	Aug. 8	220	53	70	97	44
Hamden, Conn	July 30	200	7	94	99	49
Foxon, Conn	Aug. 5	100	24	17	59	59

The data from Milton, Belmont, and Saugus show a rather high percentage of parasitization throughout the season in 1934 and 1935, and the one collection made in 1936 indicates that the same condition prevailed a third year. Collections made in the vicinity of New Haven also showed high parasitization. In 1935 the collections made about July 10 at each point near Boston showed a decided drop in parasitization. A possible explanation of this is that the overwintering *Schizonotus* had died and only a few of the first-generation adults had issued and begun oviposition. The last of the female parasites that hibernated at the laboratory died on July 1.

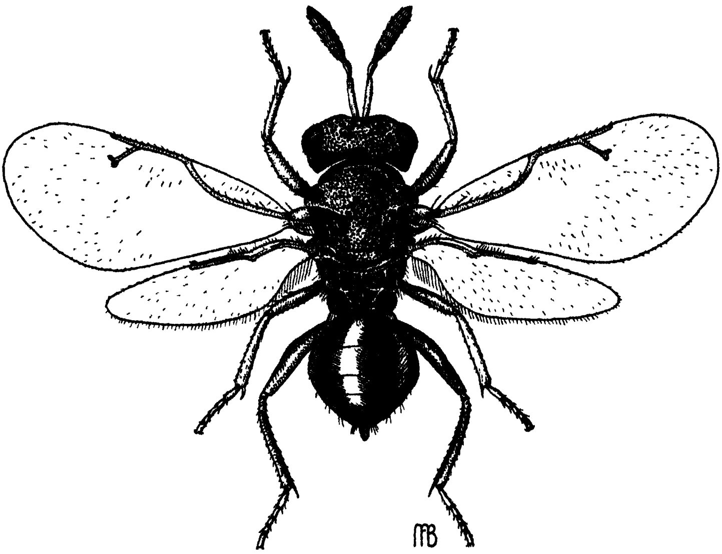

FIGURE 1.—*Schizonotus sieboldi.* Adult female.

TABLE 2.—*Average percentage of parasitization of Plagiodera versicolora by Schizonotus sieboldi in 1934 and 1935*

Place	Year	From all collections	From collections July 11 to Aug. 19[1]
		Percent	*Percent*
Milton, Mass.	1934	73	73
	1935	58	68
Belmont, Mass.	1934	59	59
	1935	57	71
Saugus, Mass.	1934	55	55
	1935	50	60

[1] These figures may furnish a more accurate basis for comparison of the 2 years than those in the preceding column, for collections were not begun until July 12 in 1934, and parasitization becomes somewhat higher as the season progresses.

In spite of the high degree of parasitization, the intensity of the host infestation at Milton, Belmont, and Saugus remained severe for 3 years and appeared to be about the same each year. The trees at Milton and Belmont were entirely browned, practically every leaf being skeletonized, and at Saugus feeding was noticeable. The three collections in the vicinity of New Haven also showed a high degree of parasitization. Feeding was severe at Hamden and West Haven, but hardly noticeable at Foxon. The percentages of parasitization were very nearly the same at all points, and the fact that feeding was less severe at Saugus and Foxon cannot be attributed to the work of the parasite unless the host populations at these points were considerably lower throughout the season. It will, of course, be necessary to devise a method of accurately measuring the host population before the true relation of this parasite to its host can be judged. Apparently, though, a severe infestation can persist for 3 years in spite of relatively high and fairly constant parasitization.

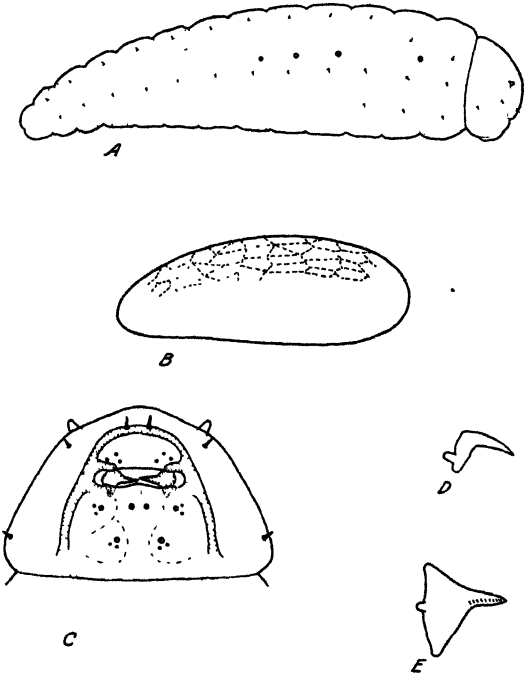

FIGURE 2.—*Schizonotus sieboldi*. *A*, First-instar larva, lateral view; *B*, egg; *C*, head of first-instar larva, ventral view; *D*, first-instar mandible; *E*, last-instar mandible.

DESCRIPTIONS

ADULT

The adult (fig 1) was originally described under the genus *Pteromalus* by Ratzeburg (*8, p. 230*) in 1852.

EGG

The egg (fig. 2, *B*) averages 0.42 mm long and 0.15 mm wide. It is cylindrical, rounded at the ends, and slightly widened at the cephalic extremity. The chorion is shiny white, with a slightly roughened area at the micropyle. One side is very finely reticulated. The sculpture is similar to that figured by Smith (*11*) for *Perilampus chrysopae* Crawf. and by Parker (*7*) for *P. tristis* Mayr, although in the eggs of those species the entire chorion is sculptured.

LARVA

In general outline and morphological characters the larva bears a striking resemblance to the pteromalids *Dibrachys cavus* (Walk.), described by Parker (*7*), and *Habrocytus cerealellae* (Ashm.), described by Noble (*6*). It belongs to Parker's group 2.

There are five larval instars. The first and the last are distinct, but the intermediate instars differ little from one another except in size.

FIRST INSTAR

The first-instar larva (fig. 2, *A*) measures about 0.45 mm in length and 0.14 mm in width. Its shape is typical of ectoparasitic chalcidoids. It has a well-defined head and 13 body segments that are about equal in length. Each segment bears a few rows of extremely minute cuticular spines on both the anterior and the posterior margins. In addition, each side bears the following sensory spines: One near the median dorsal line and one near the median lateral line of each of the first 11 segments, and one near the median ventral line of each of the first 3 segments. The sensory spines are so minute and so difficult to distinguish from the cuticular spines that the complete row along the median lateral line was not distinguished in any one specimen.

The head (fig. 2, *C*) is thimble-shaped and lightly sclerotized. The distinguishing characters are indicated in the figure. There is, however, another pair of sensory spines on the front of the head between the antennae. The mandibles (fig. 2, *D*) are simple, sickle-shaped, sclerotized hooks 0.02 mm long.

The respiratory system consists of a pair of lateral trunks united anteriorly in the first thoracic and posteriorly in the eighth abdominal segment. There are four pairs of open spiracles, a pair on the second thoracic and on each of the first three abdominal segments. They measure about 0.005 mm in diameter. Rudimentary spiracular branches are present in the third thoracic and the fourth to eighth abdominal segments, although there are no open spiracles on these segments. The spiracular branches give rise to several dorsal branches in each segment. The main ventral branches arise from the lateral trunk near the base of the spiracular branches. The head is supplied from branches arising in the first thoracic segment, and the last two abdominal segments receive branches from the longitudinal trunks in the eighth abdominal segment.

The digestive and nervous systems of this instar were not studied.

SECOND INSTAR

Second-instar larvae average 0.74 mm long and 0.38 mm wide. They are similar to the first instar, but the mandibles are shaped like those of the full-grown larva, although only 0.025 mm long. The mesothoracic spiracle is about twice as large as it is in the first instar, measuring 0.011 mm in diameter.

THIRD INSTAR

Third-instar larvae average 0.95 mm long and 0.52 mm wide. They are easily distinguished from the first two instars, for they have nine pairs of open spiracles, a pair each on the mesothoracic and metathoracic segments, and on each of the first seven abdominal segments. The mesothoracic spiracle measures 0.015 mm in diameter. The mandibles have a row of fine teeth on the inner side, and are of the same shape as in the last instar; they are 0.03 mm long.

FOURTH INSTAR

Fourth-instar larvae differ very little from those of the preceding instar. They average about 1.40 mm long and 0.77 mm wide. The mandibles measure 0.04 mm, and the mesothoracic spiracles 0.02 mm.

Fifth Instar

The fifth-instar or full-grown larvae (fig. 3, *A*) range from 2.33 mm long and 1.64 mm wide to 1.75 mm long and 1.0 mm wide, their size depending largely upon the amount of food available. Some very small individuals complete development when several larvae parasitize one host pupa. The larva is soft and flabby. It is ovoid in shape, and the color is dull gray, since the contents of the enormous midgut show through the skin. The cuticle is very thin. As in the preceding instars, there are a few rows of minute cuticular spines on both the anterior and the posterior margins of each segment, and there are the same number of sensory spines as in the first instar.

The head (fig. 3, *B*) is similar to that of the first instar except in size. The antennae are tiny, paired, nipplelike bulges in the middle of round, slightly raised areas, the antennal foramina.[4] Just above the epistoma two small oblong areas, the dorsal tentorial marks, may be distinguished. The labium bears six pairs of tiny circular sensoria. The mandibles (fig. 2, *E*) are 0.05 mm long. Along the inner margin they bear a row of minute spines.

The respiratory system is similar to that described for the first instar except that the tracheae are larger and have many more branches. There are nine pairs of open spiracles. The mesothoracic spiracle measures 0.03 mm in diameter.

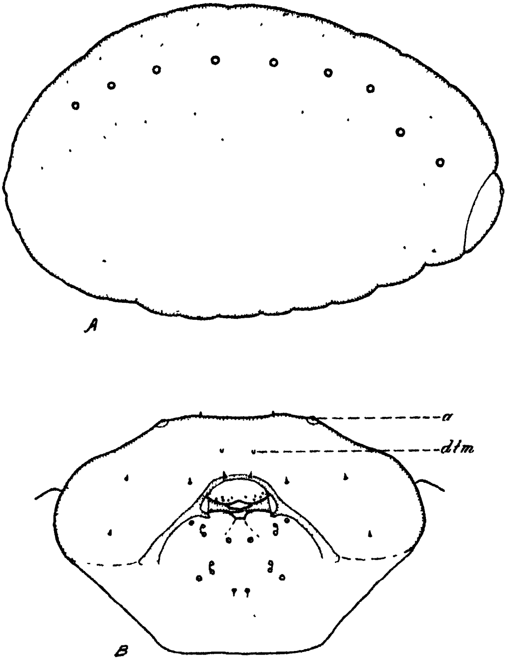

Figure 3.—*Schizonotus sieboldi*; *A*, Full-grown larva; *B*, head of full-grown larva, ventral view; *dtm*, dorsal tentorial mark; *a*, antenna.

The digestive and nervous systems are similar to those described for many pteromalid larvae. There is a pair of well-developed salivary glands, originating in a common duct on the floor of the mouth. This tube divides in the first thoracic segment. Each branch runs along one side of the larva, forming three wide undulations and ending at about the posterior margin of the midgut. There is a pair of straight malpighian tubes[5] with a branch running anteriorly along each side of the larva just above the salivary gland to about the second abdominal

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[4] The terminology is that of Vance and Smith (*12*).

[5] In a recent article Flanders calls these tubes ileac glands. Flanders, S. E., cocoon formation in endoparasitic chalcidoids. Ann. Ent. Soc. Amer. 31: 167–180. 1938.

segment, and a short posterior malpighian tube, ending just above the rectum. The ventral nerve cord is of approximately the same width throughout, showing no pronounced thickenings at the ganglia. It terminates in the sixth or seventh abdominal segment.

The imaginal buds are well developed and easily distinguished.

The fat body is very well developed. Below the median lateral line a conspicuous double row of fat cells occurs in each segment from the third to the ninth. Above the median lateral line and in the other segments the arrangement of the fat cells does not follow the segmentation so closely. There is a narrow median dorsal and median ventral region that is not covered by the fat body except in old, fully developed larvae.

PUPA

There is no cocoon. Pupae range from 1.3 mm long and 0.7 mm wide to 2.4 mm long and 1.3 mm wide. The pupa appears entirely white when first formed, but it gradually darkens, and the mature pupa is shiny black. The thin, transparent pupal skin also darkens slightly, becoming pale yellowish brown before the adult emerges.

BIOLOGY AND HABITS

SEASONAL HISTORY

Schizonotus sieboldi overwinters as an adult, becoming active in the spring and attacking the first generation of host pupae. Two or three generations are completed during the season.

The adults probably hibernate under loose bark, in debris at the base of the tree, or in similar places. In the laboratory they usually remain inactive in small groups on the sides or in the corners of the boxes provided for them, but frequently they lodge in the crevices of the cork stoppers or between the top and sides of the box.

Observations on seasonal development were made at Melrose Highlands, Mass., in 1935 by rearing in outdoor cages. Host pupae of the first generation were collected in the field on June 17, and the first generation of *Schizonotus* adults issued from July 2 to 8. The first-generation females oviposited from July 6 to 10, and the second-generation adults issued July 22 to 24. Second-generation females oviposited from July 26 to 30, and third-generation adults issued from August 12 to 14. So few host pupae were present in the field at this late date that probably most of the third-generation adults hibernated. A very few pupae were attacked and one fourth-generation adult issued on September 2.

Probably these conditions are closely approximated in the field by at least a portion of the *Schizonotus* present. However, after the issuance of the first-generation females there is constant overlapping, because the females oviposit over a considerable period and may still be ovipositing when their first progeny appear. This is equally true of the second generation, and consequently all stages of *Schizonotus* are found in the field from the time the first-generation females appear until the end of the season.

HABITS OF THE ADULT

The adults live well in confinement when fed dry lump sugar and a honey solution (1 part honey to 5 parts water) held on absorbent cotton. Several lots totaling 69 males lived an average of 7 days, with a minimum of 2 and a maximum of 12 days. Females live considerably longer. Two lots, totaling 30, isolated and held for notes on daily oviposition, lived an average of 27 days, with a minimum of 9 and a maximum of 41.

Schizonotus sieboldi adults mate readily in confinement. Ten pairs spent an average of 48 seconds in coitu.

The first eggs are deposited 2 or 3 days after a female emerges. Of 10 isolated females, 2 oviposited on the second and 8 on the third day after emergence. Oviposition takes place readily under laboratory conditions. The egg is laid externally, usually on the underside of the pupa. The female often paralyzes the host somewhat by inserting her ovipositor in the posterior end of the pupa, and then after 3 or 4 minutes approaches it again, this time from the front or side. Lying on her side she pushes her ovipositor, and often most of her body, under the pupa, and deposits from one to several eggs. The preferred location for eggs seems to be the thoracic region between the appendages, but often the abdomen and sometimes even the dorsum is chosen. The eggs are laid singly and are not fastened in any way, although they may adhere slightly to the host. After oviposition the female often drills a feeding puncture in the dorsum of the pupa. This sequence of behavior is often varied, and paralyzing or feeding is frequently omitted. Unmated females oviposit readily; their progeny are males.

It was noted in the field that *Plagiodera* prepupae sometimes bore eggs of *Schizonotus*, and once a well-developed *Schizonotus* larva was found on a prepupa. In the laboratory prepupae were attacked fairly readily, but a large percentage of those attacked failed to pupate, and most of those failing to pupate died without producing *Schizonotus*. Out of 103 prepupae attacked, 37 formed pupae and 66 failed to pupate. Of the 37 that formed pupae, 34 produced *Schizonotus*. Of the 66 that failed to pupate 61 produced nothing and 5 produced *Schizonotus*. The parasite may, therefore, occasionally complete its development on prepupae.

Rather extensive observations were made regarding the reproductive capacity of female *Schizonotus*. For 2 consecutive years 15 females were isolated in 4-inch glass vials and provided with fresh pupae daily. Each day the pupae were removed, and the eggs deposited were counted. The largest number of eggs laid by any one female was 424. With the exception of 1 day, she oviposited for 36 consecutive days. The largest number of eggs laid in any 1 day was 34. The previous day she had laid 6, and the following day she laid 13 eggs. When this female died, dissection showed a number of eggs in the ovarioles, but some of the follicles were empty at their extremities. The records made the first year were inadequate, because some females were living after the supply of host material was exhausted. The second year 15 females averaged 181 eggs each.

During the winter of 1936–37 observations were made on the ability of the adults to hibernate under cage conditions. Three lots were held in glass-covered wooden boxes, measuring 9 by 5 by 3½ inches. The boxes contained pieces of porous cork, in which the insects like to crawl, and each lot was supplied with food in the form of a honey-water solution held on absorbent cotton. Lot 1 consisted of 100 males and 100 females that emerged August 12. They were held in an outdoor insectary during the period of observation. First activity was noted April 24, and an examination on May 6 showed 36 living females. All the males were dead. Between May 6 and June 8, when the first host pupa was observed in the field, 27 more females died, leaving only 9 active females. Lot 2 consisted of 200 males and 200 females that issued August 11. They were held at 62° F. until October 1 and then placed at a nearly constant temperature of 44° and a relative humidity of 70 percent. On May 6 there were 111 females living. They were then held in an inside insectary until June 8. There were 94 living on that date. Lot 3 contained 29 males and 30 females that issued the first part of July. These adults were from the first generation completed under field conditions. They were held under the same conditions as lot 2, and there were three females still alive on June 8. The heavy mortality occurring in lot 1 between May 6 and June 8 may have been caused by too close confinement during several very warm days. The insects were very active on these days, and had they been in the field they might have sought much cooler conditions. Lot 2 was held inside and was therefore not subjected to such extremes of temperature.

LARVAL DEVELOPMENT

The egg of *Schizonotus sieboldi* hatches in from 36 to 48 hours. The young larva may crawl about, but almost immediately it begins to feed on the soft underside of the host pupa. The larva feeds externally throughout its life, sucking the contents of the host pupa through the delicate skin. The rate of development depends somewhat upon the temperature, but it is usually rather rapid. From 36 to 48 hours is sufficient for each of the first four larval instars, 1 to 2 days for the last instar, 1 day for the prepupa, and about a week for the pupa. Adults usually issue in from 14 to 16 days after egg deposition, but they have been reared in 11 days in the laboratory.

NUMBER AND SEX OF SCHIZONOTUS PER HOST PUPA

Three hundred field-collected *Plagiodera* pupae isolated at the time of collection, and subsequently proved to be parasitized by *Schizonotus*, were examined for data regarding the number and the sex of the parasites. A total of 655 *Schizonotus* adults issued, or an average of slightly more than 2 per pupa. Sixty-three pupae produced only 1 parasite each, 142 produced 2, 75 produced 3, 18 produced 4, 1 produced 5, and 1 produced 6. There were 328 males and 327 females. Both sexes of *Schizonotus* were represented in most cases where more than one parasite developed on a single pupa. Out of 229 pupae, 180 produced both sexes and 49 had more than one specimen of the same sex.

SUPERPARASITISM AND SECONDARY PARASITISM

Cushman (*2*) noted that in the case of superparasitism by *Schizonotus* young larvae were found feeding on the older parasites. Such a phenomenon was not observed during this study, and it is believed to be of rather rare occurrence. As a rule several *Schizonotus* larvae feed side by side on a single host pupa. Frequently a tiny undernourished larva completes development, but it is believed that most such larvae are crowded out altogether and die of starvation.

Jones (*4*) recorded heavy parasitization of *Schizonotus* collected in the field at Milton, Mass., in 1932. Out of 84 pupae that were parasitized, *Schizonotus* issued from only 39. In the remaining 45 pupae the *Schizonotus* had been attacked by hyperparasites. Two species of hyperparasites, *Pleurotropis tarsalis* (Ashm.) and *Tetrastichus*, n. sp. (undescribed) were reared from *Schizonotus* by the writer at practically every point at which *Schizonotus* was collected. Figures on the extent of secondary parasitization were obtained from a few small collections made in 1936. They are presented in table 3.

TABLE 3.—*Secondary parasitization of field-collected Schizonotus larvae and pupae*

Place of collection	From field-collected larvae				From field-collected pupae			
	Larvae collected	Adults issued			Pupae collected	Adults issued		
		Schizonotus	*Tetrastichus*, n. sp.	*Pleurotropis tarsalis*		*Schizonotus*	*Tetrastichus*, n. sp.	*Pleurotropis tarsalis*
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Milton, Mass.	45	35	10	--------	102	65	31	2
Belmont, Mass.	54	25	23	--------	53	15	27	3
Saugus, Mass.	135	129	5	--------	148	136	12	--------
Hamden, Conn.	60	58	1	1	60	48	5	7

The data presented in table 3 indicate that at times *Schizonotus* is severely attacked by hyperparasites. Since so few collections were made in which *Schizonotus* was isolated, however, no definite conclusions regarding their importance can be made.

The life history of *Pleurotropis tarsalis* has been determined by Muesebeck and Dohanian (*5*). The species acts as both a secondary and a tertiary parasite when it attacks cocoons of *Apanteles melanoscelus* (Ratz.). It seems probable that it may develop directly upon *Schizonotus*, attacking the pupa, or upon *Tetrastichus*, if the attacked pupa already contains a *Tetrastichus* larva. It oviposits directly into its host. One or two generations are completed annually.

Tetrastichus, n. sp., also oviposits directly into its host. The parasite drills through the pupal shell of *Plagiodera* and into the *Schizonotus* larva or pupa beneath. It has also been observed ovipositing directly into a *Schizonotus* pupa. Hibernating larvae produced adults the last of May. More than one generation may develop in a season. Some first-generation larvae hibernate, but some first-generation adults issued as early as June 28 and adults of a later generation issued in September.

SUMMARY

Schizonotus sieboldi (Ratz.) is a primary parasite of the imported willow leaf beetle (*Plagiodera versicolora* (Laich.)) and some other closely allied Chrysomelidae. It is well distributed throughout Europe and the United States and is apparently present wherever the beetle occurs in this country. High percentages of parasitization have been noted over a 3-year period in the vicinity of Boston, Mass., but about the same degree of host infestation seems to have been maintained.

The immature stages of *Schizonotus sieboldi* have been described in some detail. They are very similar to those of the pteromalids *Dibrachys cavus* (Walk.) and *Habrocytus cerealellae* (Ashm.).

The winter is spent as an adult. Two and sometimes three generations develop during the summer. The females live, mate, and oviposit well under laboratory conditions. The maximum number of eggs laid by one female was 424. The eggs are laid singly on the venter of the host pupa, and the larvae feed externally. About 15 days is required from egg deposition to adult emergence. On an average two individuals develop on one *Plagiodera* pupa. The proportion of the sexes is equal.

Two hyperparasites, *Tetrastichus*, n. sp., and *Pleurotropis tarsalis* (Ashm.), have been reared from *Schizonotus* pupae. At times *Tetrastichus* is an important enemy of *Schizonotus*.

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INCIDENCE AND INHERITANCE OF POLLEN STERILITY IN THE POTATO[1]

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INTRODUCTION

Although pollen sterility effectively prevents fruiting in most of the cultivated varieties of the potato (*Solanum tuberosum* L.), seedling populations have appeared to be relatively fruitful over a period of 15 years. These observations are based on a study of the condition of seedling populations with respect to pollen viability and on tests to determine the heritability of pollen sterility.

CONDITION OF SEEDLING POPULATIONS WITH RESPECT TO POLLEN VIABILITY

INCIDENCE OF VIABLE POLLEN SELFED LINES

In 1935 and 1936 observations were made on the flowering and fruiting individuals obtained among 2,786 individuals from 101 lines selfed from 1 to 3 generations. The lines represented material of diverse origin. Of the 2,786 individuals for which data are given in table 1, 1,332 flowered and 811 set fruit. Failure to flower resulted in absence of fruit on 50.2 percent of the total population. Presumably the 60.9 percent of flowering individuals that set fruit had viable pollen in quantities adequate for fruit setting. This leaves 39.1 percent of the flowering individuals that may have had an insufficient supply of viable pollen for fruit production. When the observations for the 2 years are examined separately, it is found that of the flowering individuals 38.3 percent fruited in 1935 and 79.0 percent in 1936. The fact that fewer fruited in 1935 was probably only partly if at all due to a greater prevalence of pollen sterility.

TABLE 1.—*Flowering and fruit production in the selfed lines grown at Castle Danger, Minn., in 1935 and 1936*

Year	Families	Individuals	Individuals— Flowering	Individuals— Fruiting
	Number	*Number*	*Number*	*Number*
1935	32	1,355	593	227
1936	69	1,431	739	584
Total	101	2,786	1,332	811

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[1] Received for publication June 16, 1938. Joint contribution of the Division of Horticulture, State Agricultural Experiment Station, University of Minnesota, and the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture. Paper No. 1623 of the Scientific Journal Series of the Minnesota Agricultural Experiment Station.

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It will be noted that the percentage of flowering individuals was also smaller in 1935.

TABLE 2.—*Percentage of cross-pollinations in three different localities that set fruit in 1930 and in 1932–37*

Year	University Farm		Duluth		Castle Danger	
	Cross-pollinations	Successful cross-pollinations	Cross-pollinations	Successful cross-pollinations	Cross-pollinations	Successful cross-pollinations
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*
1930	229	24.0	245	27.4	----	----
1932	63	12.7	56	30.4	53	58.5
1933	36	0	27	22.2	51	66.6
1934	----	----	198	16.2	269	45.4
1935	----	----	59	28.8	435	39.3
1936	----	----	----	----	264	46.9
1937	----	----	----	----	305	57.4

The importance of factors other than pollen sterility in restricting fruit set is indicated by the percentage of hand cross-pollinations that set fruit in different years and at three [2] locations, as shown in table 2. The percentage of hand-pollinations that set fruit ranged from 0.0 to 66.6. These extremes were obtained in 1933 on identical samples; one grown at University Farm, the other at Castle Danger. In general the percentage of hand-pollinations that set fruit was higher at Castle Danger than at Duluth, and was lowest at University Farm. These differences may have been associated with temperatures which were more favorable to the growth of the potato at Castle Danger than at Duluth and least favorable at University Farm. The results indicate that after flowers are pollinated with viable pollen, subsequent environmental conditions may influence the set of fruit. These environmental factors apparently affect the fruit set at the time of pollination and for a few days thereafter. This is indicated by the percentage of hand cross-pollinations that set fruit in each of three periods in 1936 at Castle Danger.

TABLE 3.—*Percentage of cross-pollinations that fruited in each of three periods at Castle Danger, Minn., in 1936*

Period	Cross-pollinations	Successful crosses		Clusters pollinated	Clusters setting fruit
	Number	*Number*	*Percent*	*Number*	*Percent*
July 14–22	123	64	52.0	197	43.1
July 23–31	90	53	58.9	130	54.6
Aug. 6–10	51	7	13.7	82	9.8

The data presented in table 3 show that the conditions which caused the low set of fruit from August 6 to 10, apparently had not influenced the set of fruit during the period from July 23 to 31. The percentage of hand-pollinated individuals that set fruit was lower in all three periods than the percentage of individuals that set fruit in the selfed lines in the same plot as shown in table 1 for 1936.

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[2] These locations were: University Farm, St. Paul, Minn.; a plot at Duluth about 6 miles inland from Lake Superior; and plots at Castle Danger immediately adjacent to the north shore of Lake Superior and on a point extending into the lake.

The observations on the selfed lines may now be evaluated. Of the 2,786 observed individuals, 1,454 failed to flower, and of the 1,332 that flowered, 811 set fruit. From these results it would appear that 60.9 percent of the population had a supply of viable pollen adequate for natural fruit setting. The evidence presented on the influence of environmental factors in restricting fruit set on hand-pollinated flowers suggests that the failure of 39.1 percent of the flowering individuals in the selfed lines to set fruit may have been due to these factors rather than to an insufficient supply of viable pollen for natural fruit production.

INCIDENCE OF VIABLE POLLEN IN A GROUP OF CLOSELY RELATED LINES

It has been shown that while fruit setting may give some approximation of the degree of pollen sterility among the flowering individuals of a population, its value is limited by the inability to eliminate the influence of other factors that may cause nonfruiting of flowering individuals. The observations suggested the possibility that relatively few of the seedlings from seed of naturally set fruit may have had an insufficient supply of viable pollen for fruit production. To obtain information on this point a study was made of the pollen and fruit setting in 514 of 1,431 individuals from 69 families representing the F_1 to the F_4 generations of a cross. The condition of the pollen was recorded in percent stainable with acetocarmine. It is probably safe to conclude that pollen which does not stain is nonfunctionable. The association between stainable pollen and fruit production is presented in table 4.

TABLE 4.—*Association between the average percentage of stainable pollen and fruiting in 514 seedlings from 69 families constituting the F_1 to the F_4 generation, inclusive, of a cross*

Stainable pollen (percent)	Plants observed	Plants setting fruit		Stainable pollen (percent)	Plants observed	Plants setting fruit	
	Number	*Number*	*Percent*		*Number*	*Number*	*Percent*
0.0–5.0	60	33	55.0	60.1–70.0	80	76	95.0
5.1–10.0	25	19	76.0	70.1–80.0	53	52	98.1
10.1–20.0	42	33	78.6	80.1–90.0	26	26	100.0
20.1–30.0	38	29	76.3	90.1–100.0	1	1	100.0
30.1–40.0	44	35	79.5				
40.1–50.0	64	60	93.7				
50.1–60.0	81	75	92.6	Total or average	514	439	85.4

Of the 60 seedlings that had between 0 and 5 percent stainable pollen, 55 percent set fruit, and of the 25 seedlings that had between 5.1 and 10 percent of stainable pollen, 76 percent set fruit. There was a gradual increase in the number of individuals setting fruit as the percentage of stainable pollen increased. While the association between amount of stainable pollen and the number of fruiting individuals found in the two lower classes was not unexpected, the significance of the association in the higher classes is not clear. The fruiting observed in individuals of the group with 0 to 5 percent stainable pollen suggests that under the conditions of these observations most of the stainable pollen was probably viable.

It will be noted that 439 of the 514 individuals, or 85.4 percent, set fruit. This is slightly higher than the 79.0 percent obtained in the selfed lines in 1936. Since 33 of the 60 individuals having between

0 and 5 percent stainable pollen set fruit it would appear that 27 individuals, or 5.3 percent of the total population, may have had an insufficient supply of viable pollen for fruit setting. The observations tend to support the view that pollen sterility plays a relatively minor role in restricting fruiting in seedlings from seed of naturally set fruit.

The influence of environmental factors on the viability of pollen is indicated by the percentage of stainable pollen observed in 10 clones, for each of two periods (table 5). A significant difference in viability is indicated between the two periods for all but one clone. The decrease in stainable pollen noted in the period from June 26 to July 5 was associated with a distinct rise in temperature and a large amount of flower abscission. The data are in harmony with the observations of Stow[3] and support his view that pollen viability is modified by temperature.

TABLE 5.—*Stainable pollen observed in 10 clones during two periods in 1933 at University Farm, Minn.*

Variety or stain of clone	Stainable pollen observed from—		Variety or stain of clone	Stainable pollen observed from—	
	June 12 to 21	June 26 to July 5		June 12 to 21	June 26 to July 5
	Percent	*Percent*		*Percent*	*Percent*
Green Mountain	1.8	0.6	11-1-12	24.0	18.8
Katahdin	31.4	11.5	40-2-2	23.5	2.3
4-20-1	42.5	47.4	41-1-1-8-1	26.8	10.2
4-39-1-1	17.2	.0	66-5	26.0	.2
5-4-1-2	24.2	.0	*Solanum demissum*	17.6	1.2

It has been observed that clones may vary in percentage of stainable pollen between periods within a season, the amount of variation depending upon environmental conditions. Significant variation between paired flowers of a clone have also been observed. Table 6 gives the percentage of stainable pollen observed in each of two concurrently developing buds on each of four clones. The percentages are based on 2,500 observed pollen grains in each flower. While the variation between flowers of the same clone is distinctly less than that between clones, the differences between paired flowers is significant for Selections 1 and 3. These results are in agreement with those of Longley and Clark,[4] who mention numerous instances of observed variation in tetrad formation between flower buds of the same clone.

The variations observed suggest the desirability in inheritance studies of making progeny tests in classifying the individual segregates.

TABLE 6.—*Percentage of stainable pollen in two paired, freshly opened flowers from each of four clones*

Flower No.	Selection 1	Selection 2	Selection 3	Chippewa
	Percent	*Percent*	*Percent*	*Percent*
1	2.5	34.8	35.0	0.0
2	12.3	32.7	20.8	.1

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[3] STOW, ISAMU. A CYTOLOGICAL STUDY OF POLLEN STERILITY IN SOLANUM TUBEROSUM L. Japan. Jour. Bot. 3: [217]–238, illus. 1927.

[4] LONGLEY, A. E., and CLARK, C. F. CHROMOSOME BEHAVIOR AND POLLEN PRODUCTION IN THE POTATO. Jour. Agr. Research 41: 867–888, illus. 1930.

INHERITANCE OF POLLEN STERILITY

In 1924 a cross was made between the Lookout Mountain variety and an unknown variety found as a mixture in a commercial field of Irish Cobbler. Both parents were known to have a moderate amount of fertile pollen. The F_1 plants were relatively fruitful and seed of 45 families of the F_2 to the F_4 generations was accumulated during a period of 13 years. The 45 families were grown in 1937 and the percentage of stainable pollen of a number of individuals in each family was determined.

With respect to their production of stainable pollen, the 16 individuals of the F_1 family fell naturally into 3 groups, as follows: 5 with 0 to 17 percent of stainable pollen, 6 with 30 to 46 percent, and 5 with 70 to 77 percent. Three types of segregation were obtained from the F_1 plants. Four gave progenies ranging in percentage of stainable pollen from high to medium. One appeared to be segregating for high, medium, and low, and two for medium and low. The percentage of stainable pollen observed in each plant is given in table 7. It will be noted that in the progeny of F_1 plant 5-8, there is one plant with no stainable pollen. It is possible that this F_1 plant was segregating for high, medium, and low stainable pollen plants. It will also be noted that a plant from 5-5 gave a high percentage of stainable pollen as compared with the other segregates observed in the family. The three types of segregation observed in the F_2 families harmonize with the observed natural grouping of the F_1 phenotypes. The F_3 and F_4 families are ranked in table 8 according to their mean percentage of stainable pollen.

TABLE 7.—*Types of segregation for percentage of stainable pollen obtained from F_1 plants from a cross of Lookout Mountain × Minnesota Accession 123*

F_1 plant No.	Stainable pollen in F_2 segregate No.—															Individuals	Mean percentage
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
	Pct	*Pct*	*Pct*	*Pct*	*Pct*	*Pct*	*Pct*	*Pct.*	*Pct*	*Pct*	*Pct*	*Pct*	*Pct*	*Pct*	*Pct*	*Number*	
5-2	80	69	65	65	64	62	59	59	57	54	52	45	41	36	--	14	57.7
5-10	86	83	81	77	63	49	42	40	25	15	--	--	--	--	--	10	56.1
5-3	78	72	58	56	51	41	29	20	--	--	--	--	--	--	--	8	50.6
5-8	72	70	63	59	59	58	56	51	47	40	40	33	30	29	0	15	47.1
5-16	65	55	38	38	14	1	--	--	--	--	--	--	--	--	--	6	35.2
5-5	73	18	12	9	7	3	2	0	0	0	--	--	--	--	--	10	12.4
5-4	37	22	9	5	4	2	0	0	0	0	--	--	--	--	--	10	7.9

Table 8.—*Types of segregation for percent of stainable pollen obtained in F_3 and F_4 families from a cross of Lookout Mountain × Minnesota Accession 123*

Group	Parent No.	Stainable pollen in progeny No.—																	Individuals	Mean percentage stainable pollen
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17		
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Number*	
1	5-10-1-5	89	85	82	78	76	74	64	63										8	76.4
	5-4-1-1	79	71	71	70	70	69												6	71.7
	5-10-2	86	83	83	83	73	70	69	68	61	48	45							11	69.9
	5-14-5	91	89	79	57	17													5	66.6
	5-3-1	80	78	76	62	60	60	59	59	58									9	65.8
	5-14-5-1	83	77	72	58	57	54	28											7	61.3
	5-14-15	85	71	65	59	57	53	50	50	44									9	59.3
	5-14-16	72	67	59	51	47													5	59.2
	5-15-1-4-1	85	74	70	65	1													5	59.0
	5-10-1-3	86	73	55	48	30													5	58.4
	5-10-1-4	91	86	83	83	83	79	79	70	61	60	22	7	4	0				14	57.7
2	5-10-3-23	76	58	56	56	54	49	5											7	50.6
	5-6-1-1	87	84	76	68	65	56	56	54	51	30	23	20	16	13				14	49.9
	5-4-3-6	67	63	62	6														4	49.5
	5-14-2	74	73	61	56	55	42	38	35	0									9	48.2
	5-1-1	75	70	69	67	60	56	56	54	53	53	40	38	29	21	17	11		16	48.1
	5-2-5	64	58	37	32														4	47.7
	5-10-3	80	63	36	11														4	47.5
	5-10-1	77	65	65	61	60	56	48	47	46	42	35	34	34	28	15			15	47.1
	5-2-4	57	46	35															3	46.0
	5-14-13	64	63	62	60	52	41	33	27	11									9	45.9
	5-11-1-4	76	71	67	10	1													5	45.0
	5-10-1-1	65	65	62	56	56	53	53	51	51	50	49	47	29	29	21	17	5	17	44.6
	5-8-2	72	67	66	58	55	52	48	48	45	40	34	18	13	2	0			15	41.2
	5-8-1	71	63	48	48	45	40	40	39	39	34	25	22	21					13	41.2
3	5-2-1	74	73	56	15	13	2												6	37.8
	5-14-9	77	65	54	15	13	2												6	37.7
	5-6-2	83	75	69	69	65	53	41	31	31	13	10	2	0	0	0			15	36.1
	5-4-1	61	50	47	43	22	8	7											7	34.0
	5-3-2	64	55	54	45	16	4	0											7	34.0
	5-4-3-4	80	70	52	51	41	40	32	31	29	29	12	12	11	6	2			15	33.2
	5-14-17	66	58	47	23	3	0												6	32.8
	5-7-2	56	54	52	48	43	41	33	25	23	22	13	5	2	2				14	29.9
	5-14-14-1	62	57	23	19	18	0												6	29.8
	5-14-12	59	51	38	34	15	6	0											7	29.0
	5-14-8	60	48	34	11	8	6												6	27.8
4	5-6-1	70	51	45	45	29	25	20	13	8	2	1	0	0	0	0			15	20.6
	5-4-3-5	58	24	9	4	1													5	19.2

There are three distinct breaks in the frequencies of the means, as follows: between 20.6 and 27.8, 37.8 and 41.2, and 50.6 and 57.7. These breaks divide the 38 families into 4 groups. The segregation observed within and between these groups suggests the possibility that this may be a natural grouping and that the parents of the 38 families may have been distributed among 4 different genotypes with respect to sterility.

The results obtained from the cross suggest that pollen sterility may be inherited as a simple mendelian character. A behavior similar to that observed might be expected if inheritance in the potato is tetrasomic and a pollen lethal lacking dominance is present. Lunden[5] has supplied evidence indicating that inheritance in the potato is tetrasomic. Assuming a pollen lethal gene designated as *V* the possible types of gametes are *V*2, *Vv*, and *v*2. If the *v*2 pollen aborts, the possible genotypes are *V*4, *V*3*v*, *V*2*v*2, and *Vv*3.

Both parents of the cross were able to produce some viable pollen. Accession 123 appeared more pollen-fertile than Lookout Mountain, for it was more effective as a male parent and selfed lines of it were easier to obtain. If Accession 123 was a *V*2*v*2 plant and Lookout Mountain a *Vv*3 plant, then the three types of segregates obtained in the F_1 could be expected. The five segregates having 70–77 percent stainable pollen would be classed as *V*3*v* plants, the six having 30–46 percent stainable pollen as *V*2*v*2 plants, and the five having 0–17 percent stainable pollen as *Vv*3 plants. It will be noted from the percentage of stainable pollen observed in the plants classed as *Vv*3 that in addition to the *v*2 gametes, from 66 to 100 percent of the *Vv* gametes aborted. In the F_2 from these F_1 plants three types of segregation would be expected, and as will be seen in table 7, the F_1 plants divide naturally into three groups, based on the mean percentage of stainable pollen in their progenies. The segregation in the families, despite the small number of individuals observed, is suggestive of the types that would be expected. In the F_3 and F_4, as will be noted in table 8, the 38 families are separated into 4 groups by more or less distinct breaks in the frequencies of their mean percentage of stainable pollen. These percentages are 76.4 to 57.7 in the first group, 50.6 to 41.2 in the second, 37.8 to 27.8 in the third, and 20.6 to 19.2 in the fourth. These groups may represent segregation from the four possible genotypes as shown in table 9. In the first group all the grains would be expected to be potentially viable, although only 64.1 percent were stainable. It is possible that the shrunken grains observed in this group would have been viable except for nutritional or other causes arresting development. In the lowest group, which presumably is composed of families derived from *Vv*3 plants, the average mean percentage of stainable pollen for the two families is 19.9. This result would be expected if all the *vv* pollen, and about 80 percent of the *Vv* pollen, aborted. This is in harmony with the behavior of the *Vv*3 plants observed in the F_1, in which the percentage of stainable pollen in different plants ranged from 0 to 17 percent, showing that none of the *vv* pollen and only from 0 to 34 percent of the *Vv* pollen was stainable.

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[5] LUNDEN, AKSEL P. INHERITANCE STUDIES IN THE POTATO (SOLANUM TUBEROSUM L.) Norges Landbrukshøiskoles Åkervekstforsok Årsmeld. (1934-35) 46: 1-156. 1937.

TABLE 9.—*Probable genotype of parents of each group, the gametes and the expected segregates with the expected average percent of viable pollen from the progeny and the percent of stainable pollen observed, for each group* [1]

Group No.	Parents	Gametes	Segregates				Segregation of male gametes in progeny	
			1	2	3	4	Viable gametes calculated [1]	Stainable pollen obtained
							Percent	*Percent*
1	*V*4	*V*2	*V*4				65.00	64.1
2	*V*3*v*	*V*2 *Vv*	13*V*4	17*V*3*v*	4*V*2*v*2		48.95	46.6
3	*V*2*v*2	*V*2 4*Vv* *v*2	13*V*4	68*V*3*v*	77*V*2*v*2	16*Vv*3	33.08	32.9
4	*Vv*3	*Vv* *v*2			*V*2*v*2	*Vv*3	17.08	19.9

[1] Calculated on the basis that 35 percent of the pollen with *V*2 gametes failed to develop, and that all the *v*2 gametes and 80 percent of the *Vv* gametes resulted in aborted pollen

The fact that only 65 percent of the *V*2 pollen from *V*4 plants, and about 20 percent of the *Vv* pollen and none of the *v*2 pollen from *Vv*3 plants was stainable, gives a basis for calculating the expected mean percentage of stainable pollen in the progenies from *V*3*v* and *V*2*v*2 plants. The calculated and the observed percentages are given in table 9 and, as will be seen, the agreement is reasonably good.

DISCUSSION

In contrast to the condition prevailing in cultivated varieties of potato, the viability of the pollen in most of the hybrids of the cross studied was relatively high. Pollen from 514 of the 1,431 individuals in the cross was examined and only 60 were found to have less than 5 percent of stainable pollen. Since 33 of these 60 produced fruit, only 27 individuals, or about 5 percent of the individuals that produced flowers, can be suspected of having failed to fruit because of an insufficient supply of viable pollen. The behavior of the F_2, F_3, and F_4 families was very similar to that observed in families derived from selfing varieties and clones of very diverse origin. This general behavior in the potato suggests that the cultivated varieties, which have little or no viable pollen, may not be representative of the species with respect to production of viable pollen. It is possible that there is sufficient antagonism between fruit production and growth of vegetative organs in the potato so that selection for high yield of tubers has tended to eliminate the profuse blooming and highly fruitful types.

It should be remembered that pollen abortion is only one of the many factors responsible for low fruiting in the cultivated varieties. Some of the other factors that influence fruitfulness in cultivated varieties are restricted habit of bloom, rudimentary to weak inflorescence, ready abscission of flowers, and poorly developed stamens. Their presence lends support to the view that selection for high yield has favored the retention of genes adversely influencing sexual reproduction. On the other hand, continued sexual reproduction tends to eliminate these genes and selection is required to retain them.

SUMMARY

Observations on 2,786 individuals derived from 101 selfed lines of potato indicated that 47.8 percent flowered and 60.9 percent of the flowering individuals set fruit. Evidence from hand cross-pollinations suggests that the failure of 39.1 percent of flowering individuals to set fruit might possibly have been due to environmental factors.

Observations on pollen and fruit setting for 514 individuals of 69 families showed an association between amount of stainable pollen and number of individuals within the group that set seed. It was calculated that on the basis of the observations 94.7 percent of the total population had viable pollen in adequate quantities for setting fruit naturally. An association was observed between percentage of stainable pollen and changes in environmental conditions.

In studying the inheritance of pollen sterility, stainability was used to differentiate between viable and nonviable pollen. The study was confined to one cross. Progeny tests were used to classify the individual segregates. The segretation obtained may be explained by assuming the presence of a tetrasomic gene which, when present in the pollen in the homozygous condition, is lethal and in the heterozygous condition partly lethal.

SEASONAL VARIATION IN THE COMPOSITION OF BLUEBUNCH FESCUE[1]

By RALPH McCALL[2]

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INTRODUCTION

Bunch grasses have long been considered superior for fall and winter feeding, presumably because of the fact that there is little loss of nutrients during that period. There is, however, considerable variation among bunch grasses in composition and feeding value. This work was planned to show the chemical change throughout the year due to the effects of stage of maturity and weathering as an indication of the feeding value of a grass typical of the ranges in the State of Washington. The study was conducted with bluebunch fescue (*Festuca idahoensis* Elmer) (sometimes called blue bunch grass), because it is one of the most common range grasses of Washington, and because a typical stand of this grass was available.

REVIEW OF LITERATURE

SEASONAL VARIATION IN THE ORGANIC NUTRIENTS OF GRASSES

Much of the work done on the seasonal variation in composition of grasses has been with cultivated pasture grasses rather than with range grasses. Although the trend is probably slightly different in tame grasses, some of the work on these grasses is presented for comparison.

Woodman, Blunt, and Stewart (*23*)[3] reported that the nutritive value of pasture grass as indicated by chemical analysis is highest during the early part of the season, and as the plant matures, there is a gradual decline in nutritive value. Catlin (*5*) observed a similar decline in experiments with range grasses and other forages. Cundy (*6*) found that the decline is not usually quite so great in range grasses as in pasture grasses.

Considerable importance attaches to the seasonal trend in chemical composition of a forage as the amount of crude protein, calcium, and phosphorus in a plant is one of the best indexes of its feeding value according to Guilbert (*11*). Lush (*17*) observed that the season of the year and the rate of growth were of more importance than the type of vegetation in determining the crude protein and crude fiber of tame grasses. Hart and his coworkers (*13*) concluded that the dry matter of range forages varies from a protein-rich concentrate during the early vegetative stages to a poor roughage during the drought period.

Green (*10*) proved that the decline in protein content of grasses levels off at maturity, that there is little further decline, and in some

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[2] The author wishes to express his appreciation for helpful suggestions and criticisms to J. Sotola, H. Hackedorn, and E. H. Steffen of the Washington Agricultural Experiment Station.

[3] Italic numbers in parentheses refer to Literature Cited, p 615.

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cases a slight increase is observed throughout the fall and winter. He believes that the seasonal decline in crude protein does not represent an actual loss of this nutrient but may be attributed to the accumulation of greater quantities of crude fiber and other nutrients.

Capen and Le Clerc (*4*) reported that the crude fiber of tame pasture grass and range grass usually shows a seasonal trend in the opposite direction from that of crude protein.

Hopper and Nesbitt (*14*) observed that the seasonal variations in the crude-fat content of range grasses are small and exhibit little consistency. Cundy (*6*) learned that a majority of range grasses show an increase in crude fat as the season advances. Knight and his coworkers (*15*) found the reverse to be true.

Woodman and Oosthuizen (*25*) concluded that as a general rule there seems to be a slight increase in the nitrogen-free extract content of grasses as the season advances. However, Guilbert, Mead, and Jackson (*12*) found that there was a loss of nitrogen-free extract because of weathering of range grasses and forages in California.

Woodman, Norman, and French (*24*) suggested the following effects of drought on tame grasses: (1) A decided decrease in crude protein; (2) a slight increase in crude fiber and nitrogen-free extract; (3) a sharp increase in lime; (4) decreases in phosphorus; (5) pronounced moisture decreases; and (6) a decided decline in digestibility and nutritive value.

Archibald and Bennett (*2*) noticed that the amount of growth and percentage of crude protein and phosphorus in range and pasture grasses follow the rainfall curve very closely.

In a moist, "quick-growing" season, Woodman, Norman, and French (*24*) observed a greatly enhanced vegetative activity in pasture grasses. The amount of crude fat was depressed markedly, crude protein slightly, and crude fiber raised considerably. Knight, Hepner, and Nelson (*16*) stated that the composition of a species of range forage plant varies from year to year in the same location.

According to Hopper and Nesbitt (*14*) some of the factors that influence the chemical composition of a grass are (1) stage of growth, (2) amount of moisture, (3) fertility of soil, and (4) elevation. Knight, Hepner, and Nelson (*16*) considered the amount of sunshine, the temperature, and environmental conditions in preceding years other important factors. Archibald (*1*) added length of day to the list of factors affecting the chemical composition of grass.

According to studies by McCreary (*19*) the fineness or coarseness of the stems of range forages has no influence on the seasonal change in composition. Fagan and Milton (*8*) concluded that the change in composition of tame grasses is largely accounted for by the proportion of stem to leaf which gradually increases with age. They also observed that the leaf at all periods is richer in crude protein and lower in crude fiber than the stem.

McCreary (*18*) analyzed range grasses in late fall after the plants were in a dormant state and discovered that their feeding value as indicated by chemical analysis is little better than that of oat straw.

SEASONAL VARIATION IN THE INORGANIC NUTRIENTS OF GRASSES

McCreary (*19*) reported a gradual percentage increase of ash and lime in range grasses as the season advanced, but he suggested that it was probably due to an accumulation of dust. Roberts (*20*) observed that approximately two-thirds of all samples of range grasses studied over a 3-year period showed an increase in ash content as the season advanced. Watkins (*22*) analyzed range grasses in the fall and again in late winter and found that there was an average loss of about 78 percent of phosphorus and 26 percent of calcium at the latter date.

Guilbert (*11*) observed extreme seasonal variations in the content of calcium and phosphorus of range forages, and calcium-phosphorus ratios as wide as 45 to 1.

An observation by Harper and Daniel, reported by Watkins (*22*), is that after a growing season with heavy rainfall, the prairie hay of Oklahoma was low in calcium and high in phosphorus, but that after a growing season with light rainfall the reverse was true. McCreary (*19*) found a very close relationship throughout the year between the percentage of phosphorus and crude protein in range grasses.

Scott (*21*) reported that precipitation apparently had no effect upon the mineral content of range grasses and forages. However, Daniel and Harper (*7*) observed that soil moisture during the growing period is an important factor in increasing or retarding the intake of calcium or phosphorus from the soil by range plants.

According to Forbes and his coworkers (*9*) the leaves of grasses are richer in calcium than the seeds, and the seeds are richer in phosphorus than the leaves. Fagan and Milton (*8*) reported that the calcium content of leaves increases with age, whereas the reverse is true of stems.

EXPERIMENTAL MATERIALS AND METHODS

The samples of bluebunch fescue were collected every 2 weeks from an area about 100 feet square midway of a south slope on a virgin bunch-grass plot of the Washington State Agricultural Experiment Station farm. The vegetation here seemed to best represent the climax type. The soil is a deep Palouse silt loam and is well drained.

The cuttings of grass were made with a knife just above the crown of the tuft. Special care was taken to include all of the basal leaves. These cuttings did not exactly represent the way in which grass would be grazed on the range but were the closest possible approach to the true condition. Seven to ten bunches were included in each sample for uniformity. Succeeding cuttings were always taken from different bunches so that each cutting represented the cumulative growth up to that time. The first new-growth sample was obtained by clipping off all the previous year's growth the preceding fall so that the sample taken was all new growth. Official analytical procedure as recommended by the Association of Official Agricultural Chemists (*3*) was followed.

SEASONAL VARIATION IN ORGANIC NUTRIENTS

The nutrient exhibiting the greatest seasonal change in chemical composition as recorded in table 1 was crude protein. In the spring of 1932 the first sample of new grass collected on April 1 contained 25.55 percent of crude protein on a water-free basis, whereas only 2.85 percent of the sample of August 7, 1931, was crude protein.

In the 4 years of this study, the decline in percentage of crude protein continued with one exception until about the first part of August to the first part of September depending upon maturity. In 1934 there was a slight increase in crude protein in the second sample of new growth, but there was a gradual decrease in succeeding samples. At the point, when the content of crude protein leveled off, the grass was quite dry, and the seeds were almost completely shattered out. In the fall of 1932 the decline lasted a little longer than usual, probably because of the small amount of precipitation in the early fall as shown in table 2. However, growing conditions had been quite favorable in 1932, and the percentage of crude protein at the low point was higher than in the other years of the study.

TABLE 1.—*Chemical composition of semimonthly samples of bluebunch fescue (Festuca idahoenesis Elmer) (water-free basis)*

Date of sampling	Ash	Crude protein (N×6.25)	Carbohydrates		Crude fat	Calcium	Phosphorus	Ca as 1 ratio (Ca as 1)
			Crude fiber	N-free extract				
1930	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	
Oct 3	18 10	3 75	30 58	44 14	3 43	0 22	0 12	0 54
Oct. 17	18 56	4 40	30 29	43 78	2.97	24	15	.62
Oct 31	19 12	3 98	31 05	42 90	2 95	27	.14	.52
Nov 17	19 12	4 05	31 83	41 84	3 16	28	.13	.46
Dec. 3	19 38	4 08	30 77	42 81	2 96	27	16	59
Dec. 12	19 17	4 27	31 22	42 07	3 27	.30	.13	.43
Dec. 29	18 75	4 47	30 13	43 12	3.53	33	.12	.36
1931								
Jan 9	18 98	4 40	30 79	42.06	3 77	32	11	34
Jan. 23	18 73	4 87	30 90	42 25	3 25	33	12	36
Feb. 6	19 65	5 25	30 45	41 85	2.80	.31	.14	45
Feb. 20	19 96	4 52	31 43	41 50	2 59	30	13	.43
Mar 6	19 38	5 35	30 08	42 82	2 37	28	15	54
Mar. 20	18 22	4 56	32 38	43 07	1 77	.27	13	48
Do.[1]	9 08	18 65	23 48	44 66	4 13	.44	46	1 04
Apr 3	9 39	16 07	24 76	46 48	3 30	37	.38	1 03
Apr. 17	9 53	13 61	24 60	48 17	4 09	.38	.35	92
May 1	13.86	13 09	28 31	40 95	3 79	39	38	.97
May 15	12.97	11 20	30 20	42 19	3 44	37	.34	92
May 29	10 12	8 93	33 86	44 40	2 69	30	30	1 00
June 12	8 29	6 04	33.85	49 30	2 55	.24	20	83
June 26	9 24	5 99	32 00	49 76	2 99	30	.22	.73
July 10	8 85	3.65	35 91	49 13	2 46	22	15	.68
July 24	10.24	3.18	34 53	49 23	2 82	25	16	.64
Aug. 7	10 74	2.85	34 06	49.75	2 60	25	15	.60
Aug. 21	11 60	3.10	33 75	48 76	2 79	26	.16	.62
Sept. 4	12 69	3 27	30 29	50.87	2 88	23	14	.61
Sept. 18	10 98	2 97	32 72	50 94	2 39	19	.09	.47
Nov 27	11.48	3 58	35 10	47 35	2 49	24	11	.46
Dec. 11	14.05	4.19	33.41	45 50	2 85	.26	09	35
Dec. 25	18 80	4.92	31 19	43 22	1.87	.38	.10	.30
1932								
Jan. 8	16 83	4 50	33.60	43 34	1 73	27	.10	.37
Jan. 22	17 62	4 20	32.49	43.14	2 55	.29	.10	.34
Feb. 5	14 64	3 75	35.74	44 43	1 44	29	08	.28
Feb. 19	12.85	3 19	37.84	44 52	1 60	26	.07	.27
Mar. 5	19 21	4 85	31 73	42 34	1 87	.34	.11	32
Mar 18	19 13	5 06	31.76	41 73	2.32	32	12	.38
Apr 1	19 18	5 08	30 95	42.27	2.52	.33	.11	33
Do[1]	10 24	25 55	22 83	37 41	3 97	.48	.64	1.33
Apr. 15	11 48	18 72	22 42	43.13	4 25	.41	.27	.66
Apr 29	12.95	15.92	26 43	40.69	4 01	.39	.33	.85
May 13	13 80	12.17	27 67	42 56	3 80	39	.32	.82
May 27	12.83	9 54	27.19	46.93	3.51	.37	.25	.68
June 10	15.71	9.16	28 70	42.64	3.79	.41	.23	.56
June 24	15 50	7.42	29.24	44 72	3.12	40	.21	.52
July 8	16 83	5 40	29.41	45 24	3 12	37	.18	.49
July 22	15 14	4.70	29.85	46 17	4 14	35	.18	.51
Aug. 5	14.79	4.56	29.69	46.95	4.01	.34	.15	.44
Aug. 19	16 32	4.70	28.26	47.14	3 58	.39	.19	.49
Sept. 2	16 08	4.51	27 03	48.17	4.21	.44	.22	.50
Sept. 16	15 08	4 61	27.37	48.85	4.09	.40	.18	.45
Sept. 29	14 73	4 48	27.99	48 52	4 28	.40	.16	.40
Oct. 14	17 38	4.06	27.82	46.63	4 11	.33	.10	.30
Oct. 28	14.72	4 31	29.66	46 88	4.43	.36	.17	.47
Nov. 18	15 96	4.21	31.18	44.48	4.17	.37	.16	.43
Nov. 25	16.14	4.86	31.01	44.10	3.89	.36	.14	.39
Dec. 12	16.58	4.76	31.17	44.12	3 37	.36	.14	.39
Dec. 22	15.91	4.52	31.45	44.98	3.14	.27	.14	.52

[1] New growth here to end of year.

TABLE 1.—*Chemical composition of semimonthly samples of bluebunch fescue (Festuca idahoensis Elmer) (water-free basis)*—Continued

Date of sampling	Ash	Crude protein (N×6.25)	Carbohydrates: Crude fiber	Carbohydrates: N-free extract	Crude fat	Calcium	Phosphorus	Ca as 1 ratio (Ca as 1)
1933	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	
Jan. 6	18.24	4.93	30.52	43.00	3.31	.32	0.16	0.50
Jan. 20	17.18	5.31	31.83	42.05	3.63	.36	.13	.36
Feb. 3	16.53	5.76	31.93	42.84	2.94	.33	.13	.39
Feb. 17	18.42	5.16	31.22	41.82	3.38	.35	.14	.40
Mar. 3	17.87	5.13	31.51	41.83	3.66	.37	.14	.38
Mar. 17	18.13	5.35	31.68	42.34	2.50	.35	.18	.51
Mar. 31	17.98	5.18	30.71	42.77	3.38	.35	.15	.43
Apr. 14[1]	11.61	19.80	21.23	43.59	3.77	.44	.49	1.11
Apr. 28	13.52	14.90	27.40	40.11	4.04	.40	.41	1.02
May 12	12.11	12.44	25.80	46.00	3.65	.37	.38	1.03
May 26	11.90	10.62	30.10	43.94	3.45	.36	.28	.78
June 9	13.64	9.29	32.64	40.99	3.44	.34	.24	.70
June 23	13.58	6.93	30.38	46.03	3.08	.36	.24	.67
July 7	14.00	5.92	30.75	46.53	2.80	.34	.21	.62
July 23	13.86	5.46	32.01	45.54	3.13	.45	.24	.53
Aug. 4	12.50	4.11	31.97	48.30	3.12	.35	.17	.48
Aug. 18	13.68	4.38	33.27	44.91	3.76	.37	.16	.43
Sept. 1	14.10	4.30	31.03	49.19	1.38	.27	.16	.59
Sept. 16	12.66	3.63	32.56	47.61	3.54	.23	.15	.65
Oct. 1	14.36	4.37	33.22	45.09	2.96	.27	.15	.56
Oct. 13	14.26	5.29	30.01	46.74	3.70	.26	.20	.77
Oct. 27	13.95	5.84	31.63	44.74	3.84	.29	.18	.62
Nov. 10	12.62	4.53	34.15	46.37	2.33	.23	.14	.61
Nov. 24	12.27	4.32	34.88	46.38	2.15	.30	.15	.50
Dec. 8	12.01	4.51	34.91	45.88	2.69	.27	.14	.52
1934								
Jan. 5	10.82	5.11	35.43	45.82	2.82	.24	.19	.79
Jan. 19	12.07	4.56	35.65	45.23	2.49	.20	.17	.85
Feb. 2	10.20	4.51	36.14	47.16	1.99	.22	.14	.64
Feb. 16	15.02	6.73	30.85	44.34	3.06	.30	.21	.70
Mar. 2	14.10	8.90	29.48	44.06	3.46	.31	.25	.81
Do.[1]	9.96	17.40	25.96	42.38	4.30	.38	.44	1.16
Mar. 16	10.67	19.26	24.68	41.25	4.14	.37	.48	1.30
Mar. 30	11.45	15.14	27.58	42.01	3.82	.37	.42	1.14
Apr. 13	11.86	12.66	27.80	44.10	3.58	.38	.40	1.05
Apr. 27	12.45	11.13	31.15	42.89	2.40	.41	.37	.90
May 11	12.76	8.75	29.15	46.30	3.04	.42	.30	.71
May 25	13.59	7.75	29.63	45.71	3.32	.43	.28	.65
June 8	14.74	6.76	29.68	45.10	3.72	.44	.26	.59
June 22	14.63	5.82	29.39	46.50	3.86	.41	.23	.56
July 6	16.35	5.18	30.46	44.18	3.83	.46	.23	.50
July 20	15.47	5.14	29.08	46.46	3.85	.39	.22	.56
Aug. 3	16.65	4.76	29.12	45.72	3.75	.44	.21	.48
Aug. 17	15.72	4.36	28.24	47.52	4.16	.45	.19	.42
Aug. 31	14.91	4.00	29.55	47.19	4.35	.40	.17	.42
Sept. 14	15.60	3.47	29.30	47.99	3.64	.40	.15	.38
Sept. 29	14.57	4.13	30.86	46.97	3.47	.40	.13	.32
Oct. 15	17.63	4.67	27.87	46.55	3.28	.45	.16	.36
Oct. 29	17.68	4.52	28.79	45.32	3.69	.39	.12	.31
Nov. 10	17.55	4.84	29.38	44.89	3.34	.40	.14	.35

[1] New growth here to end of year

The percentage of crude protein consistently held its own with the advent of the fall rains and usually increased toward the latter part of the winter. On account of the extremely mild winter of 1933–34 (table 3) and the exceptionally heavy precipitation early in the winter, there was more growth of new grass in late winter than usual. Because of the great difficulty of removing all of the tips of the green grass without losing some of the leaves of the old grass, a small amount was included in the clippings taken during the month preceding the first new growth sampling. This is probably the main reason for the percentage increase in crude protein just before the first new grass was collected on March 2, 1934. Increases in crude protein earlier in the winter were probably caused largely by decreases in nitrogen-free extract.

TABLE 2.—*Precipitation by months for the years in which the study was conducted, and 5-year averages by months, 1930–34*

Month	1930	1931	1932	1933	1934	5-year average
	Inches	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*
January	1 90	2 30	2 66	4.48	2.77	2.82
February	3 38	2 02	1.80	3.15	.56	2.18
March	2 79	4.55	5.06	1.84	2.97	3.44
April	1.87	1 98	.85	.70	88	1.26
May	2.61	.44	1 96	85	1.49	1 47
June	.94	1 34	.24	.89	2 68	1 22
July	05	00	.31	.30	.19	.17
August	04	.00	.15	.60	.03	.16
September	94	.70	.17	1 55	.64	.80
October	1 05	1.38	2 41	3 46	2 64	2 19
November	2 49	2 08	5 00	1.67	1.85	2.62
December	1 36	3 05	1.48	7.12	2.60	3.12
Total	19.42	19 84	22 09	26.61	19.30	21.45

TABLE 3.—*Temperatures by months for the years in which the study was conducted and 5-year averages by months, 1930–34*

Item	Jan.	Feb.	Mar	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Average
1930													
	°F.	°F.	°F.	°F.	°F	°F.	°F	°F	°F	°F	°F.	°F.	°F.
Mean minimum	6 40	33 30	32 90	41.30	42.90	47 20	55 50	56.64	48 90	37 90	30 76	22 09	37 98
Mean	13.50	38 50	41 40	49 85	52.70	58 15	68 90	69 56	59 20	46 20	36 88	26.60	46.70
Mean maximum	20.60	43 60	49.90	58 40	62 50	69 10	82 40	82 48	69 60	52.40	43 00	31.19	55 43
1931													
Mean minimum	28 31	27 50	30 58	36 00	45.16	50 50	54 61	55 09	47 76	39 30	26 60	24 87	38 86
Mean	32 75	32 66	37 39	45 48	56 32	60 33	69 59	68.78	57 98	48 87	32 05	29 83	47 68
Mean maximum	37 19	37 82	44 25	54 96	67 49	70 16	84 58	82 48	68 20	58 45	37 50	34 80	56 49
1932													
Mean minimum	22 80	22 90	32 00	38 80	43 64	51.30	53 40	53 40	46 40	40 20	36 20	19 60	38.39
Mean	27 45	28 00	38 24	46 70	53 77	53 05	66 10	66 55	59 95	49 25	42 30	25 75	47 26
Mean maximum	32 10	33 10	44 48	54 70	63.90	74 80	78.80	79 70	73 50	58 30	48 40	31.90	56.14
1933													
Mean minimum	25.11	16 25	31.87	36.80	39.68	49.53	54.06	54 58	43.93	43.29	33.13	33 19	38.45
Mean	30.11	22 59	38.56	46 68	50 37	62 00	69.12	69 13	55 98	53 00	41 15	39 56	48 23
Mean maximum	35.11	28.93	45.26	56 57	61.06	74.47	84 19	85 68	68 03	63.71	49.17	45 93	58 01
1934													
Mean minimum	33 22	34 07	37.58	43 90	47.09	51 47	55 09	54 09	45 40	44 06	37 80	28 16	42 66
Mean	39 46	42.41	47.76	55 55	59 57	62.07	68 17	68 24	56.16	50 83	43 38	32 56	52 18
Mean maximum	45 71	50 75	57.94	67.20	72.06	72.67	81 26	82 39	66.95	57 61	48 97	36.97	61 70
5-year average:													
Mean minimum	23 17	26.80	32.99	39.36	43 69	50.00	54.53	54.76	46.48	40 95	32.90	25.58	39.27
Mean	28 65	32 83	40.67	48.85	54 55	61.12	68 38	68.45	57.85	49 63	39.15	30 86	48.42
Mean maximum	34.14	38.84	48 37	58.37	65 40	72 24	82 25	82 15	69.25	58.09	45.41	36.16	57 56

The heavy precipitation during the winter of 1933–34 had little detrimental effect on the mature grass as far as chemical composition was concerned. There was a slight decline in nitrogen-free extract in the latter part of the winter, but the similar decline in crude fiber and the increase in crude fat would indicate that the small amount of new grass included was largely responsible.

Although the yearly variation in composition at any one date during the growing and maturing season over the 4-year period was slight, particularly when the stage of growth was about the same, the variation in each of these years from the spring flush of new growth to maturity was very great. Of all nutrients, crude protein showed this variation most decidedly.

As would be expected, the content of crude fiber varied almost inversely with that of crude protein, but its seasonal change was not so decided. The new-growth samples were quite low in crude fiber, exhibiting an increase during maturation, but very little change after maturation. The highest percentage of crude fiber was found in the mature grass collected early in 1932 and 1934.

The precipitation in March and April of 1931 was high but there was none in July and August of that year, and there was little rainfall in May. Yields and test weights of grain grown on the college farm were very low in 1931, indicating rather poor growing conditions. The period from March to June 1933, which includes most of the growing season, was uniformly low in precipitation.

In both 1931 and 1933 there seemed to be a slightly lower percentage of leaves than in the other years as indicated by a higher percentage of crude fiber in the grass collected in these years. The maximum range in crude-fiber content was from 21.23 percent in the new-growth sample collected on April 14, 1933, to 37.84 percent in the old-growth sample collected on February 19, 1932.

According to Woodman and his coworkers (*23*) the process of lignification (production of lignocellulose) is completed at maturity. As practically all of the seeds were shattered out at this time, subsequent changes would be largely owing to the effect of weather. Bluebunch fescue is apparently quite high in the lignocelluloses as it is rather tough and unpalatable during the summer when it is dry but softens in the fall and becomes fairly palatable.

There was not a very definite trend in the content of nitrogen-free extract of the grass samples analyzed largely because of the dissimilarity of the trends of the other nutrients. Undoubtedly there was a high percentage of highly digestible sugars in the new growth as shown by digestion studies on similar grasses (*11*). A larger percentage of the nitrogen-free extract was probably composed of more complex and less digestible compounds as the grass matured (*11*).

The high period in content of nitrogen-free extract came in the summer and fall of 1931. On September 18, 1931, 50.94 percent of the sample was nitrogen-free extract. The first sample of new growth in 1932 taken on April 1 contained only 37.41 percent, which was the low point for the 4-year period. The late summer and early fall of each year was a period in which the nitrogen-free extract was high. At this period the grass was dry, and there was practically no green grass. Toward the end of the winter there was a consistent, though not marked, drop in percentage of this nutrient. Until the bluebunch fescue matured, the decline in percentage of crude protein and crude fat more than counterbalanced the increase in crude fiber and ash resulting in a percentage increase in nitrogen-free extract. After the seeds had shattered and vegetative activity ceased, there followed a gradual decline in nitrogen-free extract. The shattering of seed usually commenced about the middle of July.

The seasonal trend in crude fat was not very regular. The new-growth samples were always fairly high in this nutrient. After a slight diminution in the crude fat of the succeeding few samples, the variations were quite inconsistent. The grass collected in the winter of 1931–32 was lowest in this nutrient. During the late summer and early fall of 1932 and 1934 there was an unusually high crude-fat content. The weather was not unusual during these periods, but previous growing conditions had been favorable.

The maximum variation in crude fat was from 4.43 percent in the sample for October 28, 1932, to 1.38 percent in the sample taken on September 1, 1933.

SEASONAL VARIATION IN INORGANIC NUTRIENTS

The ash content of all these samples was quite high because it included the silica (table 1). The variation in amount would probably have been somewhat different had the ash been silica-free as the important minerals, calcium and phosphorus, decreased rather than increased with maturity.

The dip in the ash cycle came in the new-growth samples. Although there is more ash in leaves than in stems of grasses, first growth is very tender and consists largely of water and organic matter. The increase in ash during the fall and winter exhibited a rather distinct trend with a few variations, usually reaching a peak the latter part of the winter.

Samples taken during the fall and winter of 1930–31 were all high in ash, reaching a high point of the entire study on February 20, 1931. Crude fiber was lower than usual in the grass collected in this period. This indicates a high percent of leaves high in minerals and low in crude fiber.

Importance attaches to the seasonal variation in calcium because of its relation to the proper nutrition of farm animals. The first growth of the season was richest in this nutrient, with the exception of the 1934 season. In the other 3 years there was a fairly definite downward trend from the first sample of green grass until winter, followed by some recovery late in the winter.

About the most prolonged seasonal decline in percentage of calcium occurred in the bluebunch fescue grown in 1933. Possibly the exceptionally heavy precipitation in December of that year was responsible for the continued decline. The high percentage of calcium in the sample for July 23 of that year was probably because of an accumulation of dust on the grass at the time as the season was quite dry and dusty.

The trend in percentage of calcium was rather unusual in the 1934 season. The first new growth sample taken was rather low in calcium as compared with that collected in the other years. There was an increase rather than the normal decrease in total moisture in the first few samples of new grass, indicating a gradual build-up to the growth peak. There was also an increase rather than a decrease in calcium during this period.

The 1931 and 1933 bunch-grass samples were low in calcium as compared with those of 1932 and 1934. The proportion of stems, which are lower in calcium than leaves, appeared to be higher than usual in 1931 and 1933.

Analyses of semimonthly samples of bluebunch fescue indicate a definite decline in phosphorus, considerably more so than in calcium. The young grass was definitely high for the year in phosphorus. This high point was followed consistently by a rather sharp decline to maturity and then a very gradual decline until winter, when in some cases there was a slight increase for the remainder of the year. Again, stage of maturity, rather than the effect of weathering seemed to be the principal reason for the decline.

The trend in percentage of phosphorus in these samples very closely followed the trend of crude protein. Both were high in the young grass, folowed by a rather rapid decline until about the time the grass matured. After the grass matured, its content of crude protein leveled off, followed by a small increase during the fall and winter months. The percentage of phosphorus exhibited this trend in most cases, but to a lesser extent.

Undoubtedly the shattering of the matured seeds which are high in phosphorus hastened the decline, but a large part of the total decline had taken place before shattering occurred.

Although stage of maturity was the principal reason for the seasonal decline in phosphorus, there was a greater loss of this nutrient than of calcium after the grass matured. Probably the later decline was largely because of the effects of the weather. No doubt the use of mineral supplements rich in phosphorus would be most effective in alleviating mineral defficiency which might result from feeding bluebunch fescue during the late fall and winter period.

An unusually high percent of phosphorus was recorded for the new growth taken on April 1, 1932, but the sample collected 2 weeks later contained less than one-half as much. The April 1 sample also contained the highest recorded percent of calcium, but the decline in the next sample, collected on April 15, was relatively small compared with that of phosphorus. Apparently the first sample of new growth collected on April 1, 1932, was a little more immature than the first new-growth samples of other years, and the unusually heavy precipitation of the previous month probably caused a mushroomlike growth. The crude protein content for this sample was 25.55 percent, the highest recorded in the 4 years of the study.

In the unusually early season of 1934 the decline in phosphorus was somewhat more gradual than in the previous years. The period of maturation was longer in 1934 than in the other years of this study, and it was during this time that the principal decline in the percentage of phosphorus occurred.

CALCIUM-PHOSPHORUS RATIO

The ratio of calcium to phosphorus is not as important as the amounts of these minerals, but it is often the cause of wastage of the mineral present in larger quantities to conform to the smaller amount of the other.

The ratio of calcium to phosphorus in the skeleton of sheep is 1.30 to 1.00 (*23*), and about the same proportion in feed will best produce that ratio in the skeleton.

Table 1 shows that the early-spring growth was slightly richer in phosphorus than calcium, but at all other times the reverse was true. No extremely wide ratios were found, but the ratio of calcium to phosphorus for the sample taken on February 19, 1932, was 1.00 to 0.27. The highest proportion of phosphorus was contained in the new-growth sample for April 1, 1932, when the calcium-phosphorus ratio was 1.00 to 1.33.

FOUR-YEAR AVERAGE COMPOSITION

Table 4 shows an average of the water-free composition of the samples of bluebunch fescue taken over a 4-year period. Owing to the fact that samples were taken every 2 weeks instead of every 15 days, averages for the middle of the month were periodically included.

TABLE 4.—*Four-year averages of semimonthly analyses of bluebunch fescue*

[Water-free basis]

Period	Ash	Crude protein (N×6.25)	Carbohydrates		Crude fat	Calcium	Phosphorus	Ca to P ratio (Ca as 1)
			Crude fiber	N-free extract				
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	
Mature:								
First part of January	16.22	4.74	32 58	43 55	2 91	0.29	0 14	0.48
Last part of January	16.40	4.73	32.72	43 17	2 98	.30	13	.43
First part of February	15 26	4 82	33.56	44.07	2 29	.29	.12	.41
Last part of February	16 56	4.90	32 84	43.04	2.66	30	.14	.47
First part of March	18 82	5.11	31 11	42 33	2.63	33	.13	39
Last part of March	18 49	4.99	31 94	42.38	2 20	31	.14	.45
First part of April	18.58	5 13	30 83	42.52	2.95	.34	.13	.38
New growth:								
First part of March	9.96	17.40	25.96	42 38	4 30	.38	.44	1.16
Last part of March	9.88	18.96	24 08	42.96	4 14	.40	.47	1 18
First part of April	10 36	18 92	25 06	41 96	3 70	.41	.48	1.17
Middle part of April	11 12	16.20	24.01	44 75	3 92	40	.38	95
Last of April–first of May	13 20	13 76	28.32	41 16	3 56	40	.37	92
Middle part of May	12 91	11 14	28.20	44 27	3.48	39	.34	.87
Last part of May	12.13	9.21	30.20	45 24	3.24	.36	28	.78
First part of June	13.09	7 80	31 22	44 51	3 38	36	.23	.64
Last part of June	13.24	6 54	30.25	46.70	3 26	.37	.22	.59
First part of July	14.01	5 04	31 63	46.27	3 05	.35	.19	.54
Last part of July	13 68	4 62	31.37	46 84	3 49	.36	.20	.56
First part of August	13 67	4 07	31.21	47 68	3.37	.34	.17	50
Last part of August	14 34	4 13	30.88	47.08	3 57	.37	.18	.40
First part of September	14.56	3 93	28.96	48.74	3 81	.36	18	.50
Middle part of September	13 58	3 67	30.49	48 85	3 41	30	14	.47
Last of September–first of October	15.44	4 18	30 66	46 18	3 54	.32	.14	.44
Middle part of October	16 96	4 60	29 00	45 92	3 52	.32	.15	.47
Last part of October	16.37	4 66	30.28	44.96	3.73	33	.15	45
Middle part of November	16 31	4 41	31 63	44 40	3 25	32	14	.44
Last of November–first of December	14.82	4 21	32 94	45.16	2 87	29	14	.48
Middle part of December	15 45	4 43	32 68	44 39	3 05	30	.12	.40
Last part of December	17 82	4 64	30 92	43 77	2 85	.31	.12	.39

The percent of crude protein exhibited a regular decline to maturity on this basis and then remained quite constant although there was a slight rise during the winter. The upward trend in crude fiber from the low point in the new grass was not very regular, but the samples taken during the winter period contained the largest amount. The reverse was true of crude fat, which averaged high in the green grass with a rather irregular decline, but a definitely lower average, from November on through the winter. The average content of nitrogen-free extract was low in the new grass and high during the dry period of summer and early fall followed by a decline in the winter months.

The increase in percent of ash from the new growth on through the year was fairly regular. The phosphorus content declined quite regularly through the first part of the winter, leveling off somewhat in the late winter. However, the percent of calcium did not show a very regular or decided downward trend but was highest in the new growth of March and April.

TABLE 5.—*Average composition of semimonthly samples of bluebunch fescue for a 4-year period, by seasons, with standard deviations* [1]

(Water-free basis)

Season	Ash	Crude protein	Carbohydrates		Crude fat	Calcium	Phosphorus	Ca to P ratio (Ca as 1)
			Crude fiber	N-free extract				
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	
New growth	12.12±1.97	12.36±4.88	27.94±3.22	44.02±2.76	3.57±0.49	0.38±0.05	0.34±0.10	0.89±0.22
Maturing	14.02±2.35	4.38± .87	30.91±2.44	47.25±1.79	3.44± .61	.36± .08	.18± .03	.50± .08
Fall	15.98±2.44	4.41± .52	30.90±2.15	45.32±1.74	3.38± .65	.32± .07	.14± .02	.44± .12
Winter	16.78±2.76	4.82± .63	32.36±1.99	43.34±1.46	2.70± .67	.30± .04	.13± .03	.43± .14

[1] $\sigma = \frac{\sqrt{\Sigma d^2}}{n-1}$ when n is less than 25, $\sigma = \frac{\sqrt{\Sigma d^2}}{n}$ when n is 25 or more.

SEASONAL AVERAGE COMPOSITION

The analyses from table 1 were averaged by seasons and recorded in table 5. The new-growth season included all new-growth samples to the latter part of June; the maturing season included up to the middle of September; from then to the last of November was considered the fall season; and the winter season carried through to the first new-growth samples. The seasons in this grouping were not all the same length but represented more adequately the stages of growth and dormancy.

The seasonal grouping brings out more clearly the trend in the percentage of the various nutrients in the bluebunch fescue samples analyzed. As would be expected, the standard deviations were quite high for the new growth, particularly for the percentage of crude protein because of the decline in the crude-protein content during that period. There was no significant change in the average percent of crude protein during the 3 other periods.

The average ash content increased quite regularly from the new-growth period through the remainder of the year, but the increase was gradual and not highly significant, particularly after maturity. The trend for calcium and phosphorus was directly opposite from that of crude ash, and the phosphorus trend was significant. Because of the greater decline in phosphorus throughout the growing period, the calcium-phosphorus ratio grew wider, particularly between the first two periods.

The seasonal averages for percent of crude fiber show a slight rise from the new-growth period to the maturing period, followed by very little change during the fall period and then a slight rise through the winter. Because of the variations in each period, the difference between each period was not significant, but there was a significant difference between the new growth and the winter period. The averages for percent of crude fat gradually declined, but the only significant drop was from the fall to the winter period. The nitrogen-free extract percentages for the winter and the new-growth periods were lowest, with a fairly significant difference between periods.

SUMMARY AND CONCLUSIONS

Samples of bluebunch fescue (*Festuca idahoensis* Elmer) were gathered at semimonthly intervals from the fall of 1930 to the fall of 1934, and the analyses are reported in this study.

Any interpretation of a seasonal study of chemical composition of range grasses must be tempered by an examination of the weather records. The months of May, July, and August 1931 were hot and dry. The growing season of March to June 1933 was quite low in moisture, and the summer was hot. In each of these years the appearance of the bunches indicated a higher proportion of stems than in the more favorable years of 1930, 1932, and 1934. There was more of a seasonal increase in the percentage of crude fiber in 1931 and 1933. The percent of crude protein was lowest in the fall and winter of 1931.

Of all the factors causing seasonal and year-to-year variations in chemical composition, stage of maturity was the most important. Particularly did it affect the content of crude protein, causing variations from 25.55 percent in the young growing grass to 4.56 percent in mature grass about 4 months later. This indicates a variation from a protein-rich concentrate to a rather poor roughage. For the remainder of the fall and winter, there was little change in the percentage of this nutrient, and in most cases there was a small increase in the winter. This was probably because of a loss in nitrogen-free extract at this period. The inclusion of a slight amount of the tips of young grass, all of which could not be removed from the samples collected in late winter without losing some of the mature leaves, probably was largely responsible for the slight increase in crude protein at this time.

The percentage of crude fat showed no distinct seasonal variation but was usually highest in the first sample of new grass, after which the trend was variable. However, a summary of the analyses by seasons for the 4 years indicates a fairly significant downward trend as the season advanced. The amount of crude fat was moderate at most times as compared with that of many other forages.

The figures for percent of ash exhibited the opposite trend from that of crude fat, being lowest in the new growth. There was a gradual increase in ash content during the year which leveled off to quite a degree at maturity. The ash content was exceptionally high in view of the percentage of calcium and phosphorus, and there was probably a high percent of silica in the ash.

Nitrogen-free extract was rather a variable quantity, but usually the highest point was during the maturing period and before the fall rains became appreciable. The trend was downward on either side of this peak, being, as a general rule, lower in the new grass and in late winter.

The percent of crude fiber showed a more regular trend, being practically opposite that of crude protein, though not to the same degree. The greatest variation was before maturity, after which the trend was quite variable. Bluebunch fescue had a rather high content of crude fiber.

There was considerably more variation in percent of phosphorus than of calcium. In the young grass there was more phosphorus than calcium, but the decline in the former was considerably faster, continuing through the first part of the winter whereas the decline in percent of calcium leveled off more at maturity. The mature grass was rather low in both, particularly phosphorus. Undoubtedly the shattering of seeds at maturity was partially responsible for the decline in percent of phosphorus, but there was a decided decline in this nutrient before shattering began, which continued after shattering was completed.

As would be expected, the calcium-phosphorus ratio continually widened from the first samples of new growth on through the year. The wide calcium-phosphorus ratio in the mature grass and the small amount of both nutrients would indicate that a mineral supplement containing some calcium and a high percent of phosphorus would be desirable when the feed is largely limited to dry mature grass.

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THE DEVELOPMENT OF MYCOSPHAERELLA BERKELEYII[1]

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SCOPE OF THE WORK

In a previous paper[3] the writer described *Mycosphaerella berkeleyii* as a new species, outlined its life cycle, discussed the symptoms of the disease it produces on peanut (*Arachis hypogaea* L.) and its growth in artificial culture. The present paper is concerned with the cytological details of the development of the spermogonia and perithecia, which have not heretofore been reported.

SPERMOGONIA

As in the case of *Mycosphaerella arachidicola*, the spermogonia of *M. berkeleyii* begin to appear on diseased leaflets during the latter part of September or early in October and continue to develop throughout the fall and early winter. They develop in either leaf surface, but are more often hypophyllous. They originate within the bases of conidiophore stromata, in which case they are well concealed, or in separate stromata that develop after the death of the leaflets. In the latter case they are fairly conspicuous as a zone about the periphery of the lesions on the lower surface of the leaflets, while on the upper surface they may be found scattered throughout the lesion.

The fundaments of the spermogonia arise either subcuticularly or subepidermally. When they arise within conidiophore bases, they are surrounded from the beginning by a thick mass of stroma; but those originating independently of conidiophore bases consist first of loose wefts of interlacing hyphae which continue to grow more complex until a characteristic shape and size have been attained. In the latter case, the thick walls appear stromatous only after the developing spermogonium is exposed through rupture of the cuticle or epidermis.

As development progresses, cells toward the center of the young spermogonium enlarge (pl. 1, *A*), the nucleus of each cell undergoes one or two divisions and ultimately two to four uninucleate spermatia are formed in each cell. The spermatia often lie in tetrads but may appear in linear arrangement within the mother cells, and are liberated through a long sterigma on each mother cell (pl. 1, *B*). The details of spermatial discharge do not differ from that described for *Mycosphaerella arachidicola*,[4] but the thick stromatic wall and the long sterigmata (pl. 1, *B*) are distinguishing features of the spermogonia of *M. berkeleyii*.

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[2] The writer wishes to acknowledge the many helpful suggestions of Dr. B. B. Higgins of this station during the course of the work.

[3] JENKINS, WILBERT A. TWO FUNGI CAUSING LEAF SPOT OF PEANUT. Jour. Agr. Research 56: 317-332, illus. 1938.

[4] JENKINS, WILBERT A. See footnote 3.

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PERITHECIA

Perithecial fundaments begin to develop during the same period and in similar locations in the leaflets as do the spermogonia. Often they develop close together with only a thin wall separating them. In contrast to the situation in the young spermogonium, the cells toward the center of the young perithecium undergo very little enlargement. Instead, several to many deeply staining archicarps, each with an enlarged, uninucleate basal cell and a long, several-celled trichogyne, are produced. The writer was able to distinguish as many as eight perfectly formed archicarps in a single section of a young perithecium. In the youngest stage seen (pl. 1, *C*), the young archicarps are composed of but two to three cells and each of the cells is from one to four nucleate. Even such early stages show an enlarged basal cell which will ultimately constitute the fertile portion of the archicarp. At functional maturity, the basal cell of the archicarp is uninucleate, as are also the several cells of the trichogyne (pl. 1, *D*). In every case seen, the nucleus within the basal cell of a mature archicarp is considerably larger than the nuclei of the trichogyne, stains more deeply, and has a more conspicuous nucleolus. Owing to the large number of archicarps usually found in each perithecial fundament and the intricate manner in which they coil about the sterile hyphae, it is extremely difficult to obtain sections showing complete archicarps within the fundament. There is, likewise, the problem of obtaining the exterior portion of the trichogyne in its entirety. The exterior parts of the trichogynes of this fungus are quite long. Since they do not coil about in a limited area as do those of *Mycosphaerella arachidicola*, none of the writer's sections showed complete ones.

SPERMATIZATION

Since the exterior portions of the trichogynes were consistently cut off and washed away during the process of preparation of the slides or else remained in such obscure positions that an interpretation of their relationships was hazardous, the writer was unable to follow the initial stages of spermatization. There is much evidence, nevertheless, to indicate that spermatization is not merely a passive phenomenon depending altogether on chance. While free water appears to be essential to the discharge and dissemination of spermatia, the process of spermatization is further assured by the extensive elongation of the exterior portions of the trichogynes which, with a more refined technique, might be shown to actually grow into the vicinity of active spermogonia, even when their origins are relatively remote. Though, as stated above, the earlier stages of spermatization were not seen, it is

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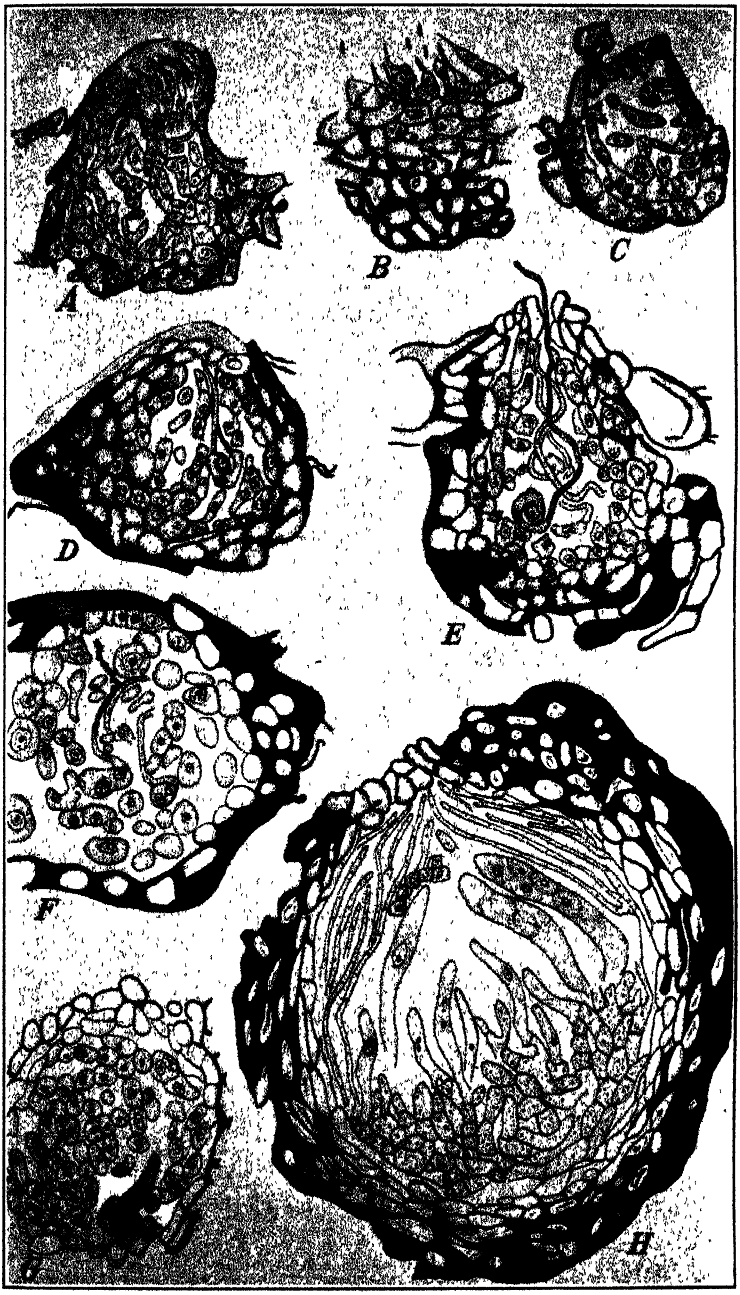
EXPLANATORY LEGEND FOR PLATE 1

A, young spermogonium of *Mycosphaerella berkeleyii* in lower surface of leaflet, showing enlargement of cells near center; *B*, portion of mature spermogonium showing origin of spermatia and thick stromatic wall, *C*, young perithecium showing origin of the archicarp. Note the several nuclei in the basal cells and trichogynes of the young archicarps; *D*, young perithecium in which one archicarp has been spermatized, while the other is not yet functionally mature; *E*, young perithecium showing several archicarps, one of which has been spermatized; *F*, portion of a young perithecium showing sperm nuclei within the basal cells of the two archicarps; *G*, portion of an old perithecium in which the one (or more) ascogonia are beginning to branch. Paired nuclei are embedded in dense masses of cytoplasm; *H*, almost mature perithecium (fixed 6–22–37) showing ascogenous hyphae, young asci, almost mature asci and periphyses. All sketches drawn to scale with the aid of a camera lucida. × 1,320.



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE

assumed that the process is like those which have previously been described.[5] The later stages show no important differences. As the sperm nucleus migrates down the trichogyne, the protoplasm and cross septa disintegrate progressively (pl. 1, *E*). Likewise, the sperm nucleus gradually enlarges during its passage down the trichogyne so that, shortly after it enters the basal cell, it is essentially as large as the basal nucelus (pl. 1, *F*); and a bit later the two nuclei become indistinguishable. Meanwhile, the portions of the trichogyne above the basal cell break down completely (pl. 1, *E*, *F*). No fusion takes place between the sperm and basal nuclei. Instead, the basal cell, or ascogonium, begins to enlarge and as it does so the nuclei divide conjugately.

MATURATION

Following spermatization the apertures at the apex of the young perithecia through which the trichogynes protruded become sealed. This feature, combined with the fact that the stromatic walls are almost impervious to the best cytological fixatives, made it extremely difficult to secure good cytological preparations of the interval between the arrival of the sperm nucleus in the ascogonium and the formation of ascogenous hyphae. Several good preparations were obtained, however, and these show that several pairs of nuclei are formed in the enlarged ascogonium before ascogenous hyphae become evident. Prior to and during the time ascogenous hyphae are being formed, the ascogonium becomes variously branched (pl. 1, *G*). During this period the nuclei continue to divide conjugately, or at least the pairs of nuclei increase in number, and the best preparations show the individual pairs of nuclei embedded in slightly denser masses of cytoplasm than is evident elsewhere in the cells (pl. 1, *G*). The writer could not be certain whether one or more than one ascogonium takes part in the development of ascogenous hyphae within a given perithecium. It is a reasonable assumption, since several archicarps within a young perithecium may be spermatized (pl. 1, *F*), that the fertile portions of the perithecia may consistently arise from several ascogonia.

By the time ascogenous hyphae become evident, the ascogonium, or ascogonia as the case might be, have undergone extensive branching and now cover the floor of the perithecium with a plectenchymatous layer. The asci arise directly or by way of croziers (pl. 1, *H*). The nuclei are usually in pairs and still embedded in dense cytoplasmic masses (pl. 1, *H*). As in the case of *Mycosphaerella arachidicola*,[5] not all the asci mature at the same time, so that ascospore discharge from a given perithecium extends over a considerable period of time.

Concurrent with the development and elongation of the asci, unequal growth stresses arise in the perithecium so that it enlarges and ultimately cracks open at the exposed apical end. During this period certain cells along the wall near the apex of the perithecium become active and form periphyses. Other sterile filaments arise down near the base of the perithecium (pl. 1, *H*). It is uncertain in some cases whether they arise from the fertile layer at the base or from the wall tissue in this area. In any event, no sterile filaments were ever found interspersed among the asci, so that the writer prefers to call them periphyses.

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[5] JENKINS, WILBERT A. See footnote 3.

Because of the scarcity of properly fixed material, no great effort was made to trace the cytological development of ascospores. From the best of the preparations studied, ascospore development differed in no important details from that described for *Mycosphaerella arachidicola.*[6]

DISCUSSION

While the general plan of development of the spermogonia and perithecia of *Mycosphaerella berkeleyii* differs in no important details from that of *M. arachidicola,* there are numerous differences in details of structure and organization that have justified this study. These differences taken together with those of spore measurements, time of discharge of ascospores, type of conidial stages, host relationships, and symptoms produced on their mutual host, abundantly justify the erection of separate species for the two fungi.

The consistency of structure in the formation of spermatia as well as the origin and development of the archicarps have been several times illustrated and emphasized in the work of Higgins and the writer,[6] as in the present work. It is not a premature assumption that work of this nature will ultimately lead to a more effective control of these and other similar fungi through a modification of their environment during certain critical stages in sexual reproduction. It is certainly true that this and other work of a similar nature will enable us to better interpret the possibilities of wide variation both in structure and pathogenicity of these fungi through the medium of hybridization.

SUMMARY

The details of structure and development of the spermogonia and perithecia of *Mycosphaerella berkeleyii* differ from those of *M. arachidicola.* The principal differences are: A greater amount of stroma surrounding the fruit bodies, longer sterigmata on the spermatial mother cells, a greater number of archicarps in the young perithecia, a greater amount of fertile tissue in the base of the perithecium, origin of periphyses, and the tardiness of ascospore discharge in *M. berkeleyii.* In addition, as stated in a previous paper, the two fungi produce different symptoms on their mutual host, differ in type of conidial fructifications, host relationships, culture characteristics, and size of ascospores.

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[6] JENKINS, WILBERT A. See footnote 3.

UNFRUITFULNESS IN THE TOMATO CAUSED BY MALE STERILITY[1]

By J. W. LESLEY, *assistant plant breeder*, and MARGARET LESLEY, formerly *assistant in genetics, California Agricultural Experiment Station*

INTRODUCTION

Some apparently healthy but unfruitful individuals usually occur in tomato fields on the Pacific coast. As the crop matures, these unfruitful plants are often conspicuous for their vegetative vigor and upright habit. Since the unfruitful plants are distributed apparently at random among the normal plants, many of them appearing healthy, disease or unfavorable soil or climatic conditions can hardly be the cause. The unfruitfulness is usually partial and often affects only a fraction of 1 percent of the plants.

ORIGIN AND CAUSE OF UNFRUITFULNESS

At Riverside, Calif., in 1933 in an F_3 population consisting of 68 plants, 1 apparently healthy but highly unfruitful plant occurred. The P_1 plants were a triploid (female) and a diploid (male); and F_1 was a trisomic having an extra H (IV) chromosome and probably another extra chromosome that was not identified. The F_1 parent was a simple trisomic, triplo-H, homozygous for the gene d_1 (dwarf), and heterozygous for *c* (potato leaf). Incidentally, inheritance of *c* in F_2 and F_3 was trisomic, showing that the locus of *c* is in chromosome IV (*H*). The F_3 population was generally healthy, matured its fruit very late in the season, and tended to be unproductive. The triplo-*H* plants were as usual especially unfruitful, but one apparently diploid plant also had this characteristic. In another F_3 family from the same F_2 parent planted the following season (1934), the diploid progeny consisted of 115 relatively fruitful plants and 8 unfruitful plants similar to the single unfruitful diploid previously observed. At the same time 2 F_3 progenies of other F_2 plants, consisting of 44 and 28 plants, contained no unfruitful diploids. In 3 F_4 populations from fruitful F_3 sibs of the original unfruitful plant, no unfruitfuls occurred, but in the fourth there were 40 fruitfuls and 10 unfruitfuls. Severe nematode infection in this season (1934) made classification uncertain, but some of these unfruitfuls seemed healthy and free from nematode symptoms.

Unfruitful diploid plants from these and related populations were self-pollinated in the greenhouse where conditions were favorable for fruit setting, but no fruit set. They readily functioned as seed parents and no difficulty was found in obtaining fruit and seeds when pollen of fruitfuls was applied.

Other unfruitful plants at Riverside and San Jose, Calif., proved to be triploid or rarely simple trisomic or tetraploid. The unfruitful plants discussed in this paper did not resemble any known chromosome mutant. Although their origin from a double trisomic one extra

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[1] Received for publication, July 18, 1938. Paper No. 394, University of California Citrus Experiment Station and Graduate School of Tropical Agriculture

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chromosome of which was not identified and the general unfruitfulness of trisomics suggested some visible chromosomal irregularity, these unfruitful plants proved to have the normal diploid chromosome number and distribution.

The nonabortion of the anthers and the nearly normal amount of pollen were not especially suggestive of a male-sterile gene mutant. However, germination tests were made with mature pollen of fruitfuls and unfruitfuls in van Tieghem cells. In 15-percent cane sugar alone practically no germination occurred, but with two or three stigmas added, as a rule about 10 to 25 percent of the grains of normal plants germinated in a few hours. Only from 0 to about 0.5 percent of the pollen grains of unfruitful plants germinated, and pollen-tube growth was much slower.

POLLEN CHARACTERISTICS

In acetocarmine smears, mature pollen of fruitful and unfruitful plants looks much alike, although the pollen of unfruitful plants is somewhat smaller and more alveolar than that of fruitful. In a dilute solution of iodine in potassium iodide, however, the two kinds of pollen were very different. Pollen smears from *mature* flowers of fruitful plants consist of numerous large, clear, pale-yellow grains, some smaller alveolar grains, and some empty grains. In diseased plants, or after very hot weather in healthy plants, the number of empty grains is greatly increased. The latter are indicative of early abortion of pollen. Anthers from open flowers of unfruitful plants very often look more waxy and paler yellow than mature anthers of fruitfuls. Pollen from the anthers of fully opened flowers of unfruitfuls, when stained in iodine, shows that most of the pollen cells contain starch (fig. 1). A very few grains have no starch; many contain a great many large, black starch grains. Smears from mature anthers of unfruitfuls appear grayish to the naked eye; those of fruitfuls a clear yellow. If pollen from slightly unripe anthers of fruitfuls is stained with iodine, the smear is like that from mature flowers of unfruitfuls. In other words, normally the starch in young pollen changes by hydrolysis, presumably to dextrins, as the pollen matures. This process is, in some way, arrested in the unfruitful plant. This arrested development has been found to be typical of unfruitfuls at all seasons. Pollen of F_1 hybrids between unfruitful (female) and fruitful (male) cannot be positively distinguished from that of the fruitful parent. Evidently the unfruitfulness is due to a form of male sterility in which pollen development is arrested and almost all the pollen is nonviable.

The most obvious characteristic of the male-sterile type is unfruitfulness (fig. 2). It is possible that other differences might be found in populations that are otherwise more nearly homozygous. Unfruitfulness is seldom, if ever, total. In the progeny of C337–1, with fruits from open pollination, the average weight of 8 fruits from a male-fertile plant was 41 gm., and of 12 fruits from three male-sterile plants, 28 gm. The corresponding average number of seeds was 59 and 24, respectively. In the progeny of C324–2, the average weight of 55 fruits from three male fertiles was 26 gm., and of 8 fruits from two male steriles, 19 gm. The average number of seeds was 36 and 19, respectively. Accordingly the fruit weight of the male fertiles was about 50 percent greater and the number of seeds about double that of the male steriles.

In some progenies, the male steriles can be picked out with certainty by their outstanding unfruitfulness; but in others, owing to gradations in fruitfulness, they can only be distinguished from the less fruitful male-fertile plants by the pollen test. Prior to 1935 the plants were classified only for fruitfulness without applying the pollen test, and some of the unfruitful plants may have been male-fertile. These

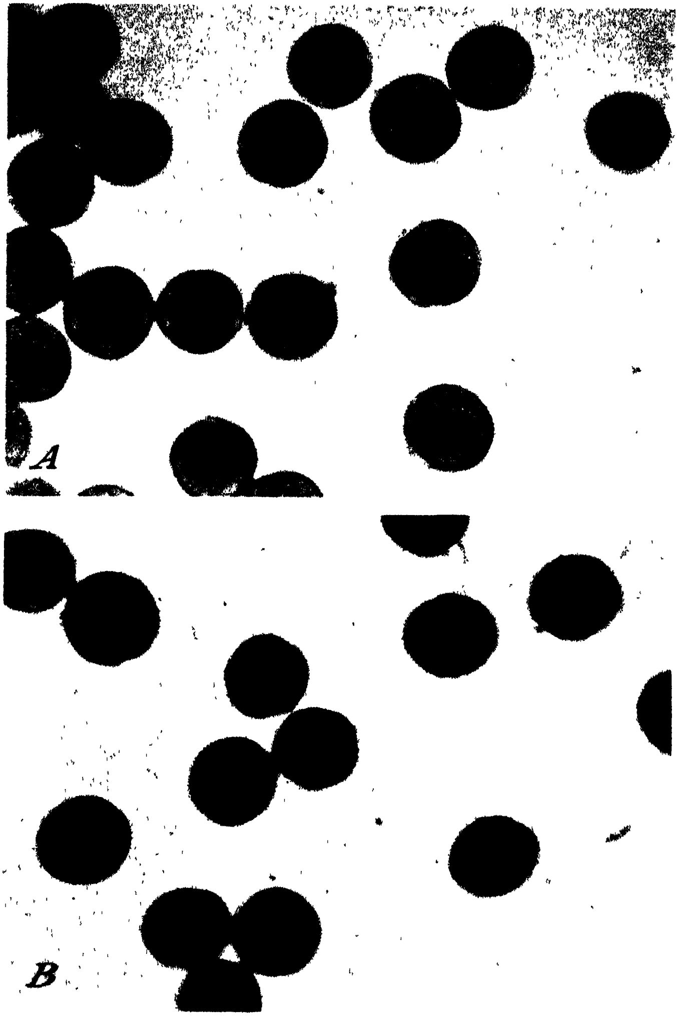

Figure 1.—Male-fertile (*A*) and male-sterile (*B*) tomato pollen stained in aqueous iodine-potassium iodide solution. × 593.

earlier data are not included in table 1. In 1935 and 1936, as shown in table 1, the pollen of all plants that were either unfruitful or low in fruitfulness, and in 1937 the pollen of every plant, was tested in iodine; and in doubtful cases three or four pollen examinations were made. An attempt was made to correlate degrees of fruitfulness with the quality of pollen determined in iodine, but no close correlation was found. In only two cases unfruitful plants proved to have normal pollen. Both of these plants subsequently set fruit readily from selfing in the greenhouse.

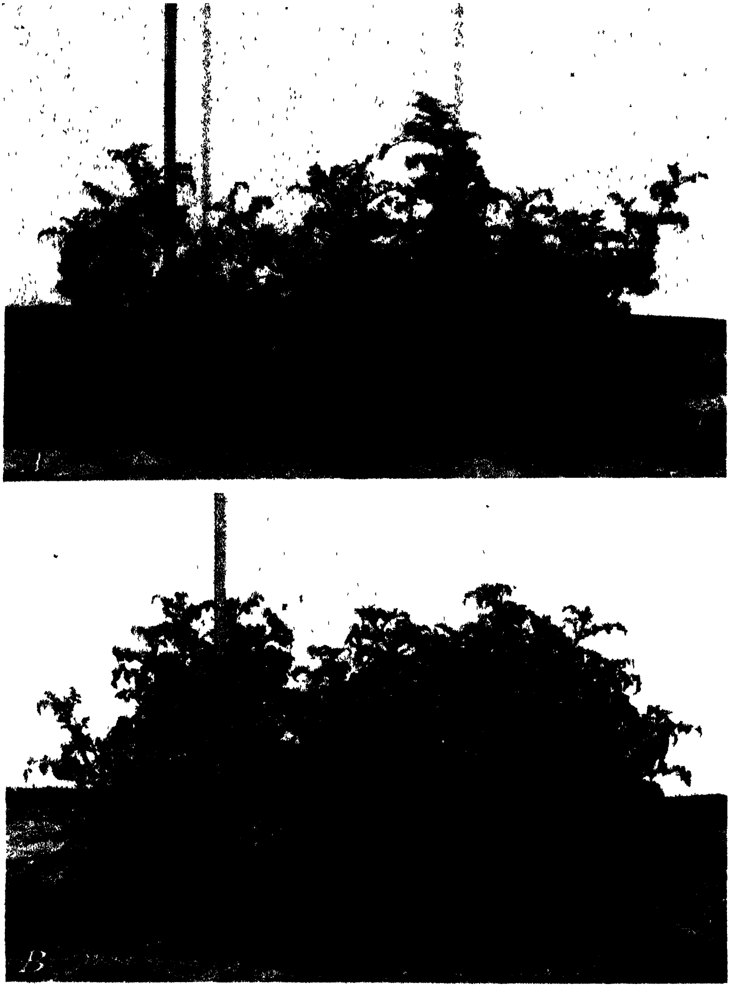

FIGURE 2.—Male-fertile (*A*) and male-sterile (*B*) tomato plants showing difference in fruitfulness. Photographed November 1, 1936.

TABLE 1.—*F_2 progeny of male sterile × male fertile and backcross progeny of male sterile × (male sterile × male fertile) tomatoes, 1935–37*

Parents	Progeny						
	Observed		Expected [1]			Plants whose pollen was examined by iodine test	Recorded [2]
	Male fertile	Male sterile	Monohybrid	Dihybrid	Trihybrid		
F_1, selfed	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
C307-3	102	16	88. 5.29. 5	111 7		39	73
C322-2	68	1		65.4	68·1	3	64
C324-1	63	6		65.4		11	32
C324-2	31	2		31.2		33	30
C337-1	175	15		178·12		42	91
C339-1	249	15		247·17		99	87
C328-1	36	3		37.2		39	36
C342-1	59	5		60 4		64	59
C342-2	31	1		30 2	31 5 0 5	32	63
C338-1	93	19	84 28	105 7		112	69
Backcrossed:							
C218-8-1-4-1 × C322-2	31	6		28·9		13	34
C218-8-1-1 × C324-1	5	0		4·1		0	9
C218 8 1-4 × C327 3	118	21		104 35	122:17	91	86
C337 1-2 × C328-1	41	21	31 31	46 5·15 5		62	77
C337-1-2 × C324-3	17	14	15 5 15 5	23·8		31	38
C337-1-7 × C324-3	18	2		15:5		20	91
C337-1-7 × C324-3 (limited pollination)	7	4		8 3		11	79
C337-1-4 × C324-3	22	5		20·7		27	100
C337-1-4 × C324-3 (limited pollination)	16	1		13 4		17	63

[1] Computed as a rule to the nearest whole number.

[2] This column gives the percentage of seeds planted which produced plants that were recorded as to pollen fertility.

Seeds were planted from fruits of male-sterile plants carrying recessive genes such as d_1 (dwarf), c (potato leaf), and a_1 (green stem), which grew adjacent to plants carrying the dominant alleles. Evidently any resulting seedling having a character dominant to the corresponding character of the male-sterile parent resulted from cross-pollination, and a seedling not having such a dominant character may have resulted from selfing. Among 152 plants from male-sterile parents, about 28 percent had characters dominant to those of the mother plant. Clearly a large proportion of the seeds resulted from cross-pollination. Although in the greenhouse attempts at self-pollination have always failed, conceivably some self-fertilization might occur if the pollen of male-sterile plants were mixed with a small proportion of fertile pollen.

GENETICS OF MALE STERILITY

All F_1 plants from P_1 male steriles pollinated by five different unrelated male-fertile parents were self-fruitful, and all those tested had normal pollen. Male sterility was completely recessive. The earlier (1933 and 1934) data suggested that male sterility was not a simple recessive. The ratios among the F_2 progeny of male steriles by unrelated male fertiles grown in 1935–37 are shown in table 1. All except the F_2 from C338–1 closely approach the dihybrid ratio, 15:1. The conformity of these F_2 ratios (table 1) to the expectation on a 15:1 basis seemed close enough to justify summation. In all F_2 families, excepting that from C338–1, the ratio of male fertile to male sterile was 814:64; and the expectation on a 15:1 basis is 823:55 ($X^2=1.6$, $P=0.2$). The F_1 and F_2 data therefore indicate that male sterility probably depends on two recessive genes. Adopting the symbols used in maize, the genes are termed ms_1 and ms_2. Male-sterile plants are homozygous for both these recessive genes, and male-fertile plants contain either one or both of the dominant alleles MS_1 and MS_2.

Four of the five different male-fertile (P_1) parents that were unrelated to the male-sterile plants in these crosses may have been homozygous for one or both of the dominant alleles MS_1 and MS_2. In the F_2 from C339–1, the ratio was 250:15, or very nearly 15:1, but in the F_2 family from C338–1, a plant which originated from another male sterile crossed with the same male fertile 35.020.1, the observed ratio, 93:19, is distinctly nearer 3:1 ($X^2=3.9$, $P=0.05$) than 15:1 ($X^2=22$, $P=0.001$). The occurrence of 3:1 and 15:1 F_2 ratios from the same male-fertile P_1 parent, 35.020.1, suggests that it was heterozygous for at least one of the male-sterility genes although unrelated to the male-sterile line. Twenty F_1 plants of the same parentage as C338–1, grown in the greenhouse, were all male fertile, although the pollen contained more alveolar grains than the typical male fertile. Probably the P_1 plant 35.020.1 was $MS_1ms_1MS_2ms_2$.

Two male-fertile F_1 plants, C324–1 and C322–2, which from selfing gave 63:6 and 68:1 ratios, were backcrossed with male steriles C218–8–1–1 and C218–8–1–4–1, and gave ratios of 5:0 and 31:6, respectively. Two other backcrosses, C337–1–7 × C324–3 and C337–1–4 × C324–3, gave 25:6 and 38:6 ratios. These agree fairly well with the 3:1 ratio expected from a backcross of a double recessive male sterile with a male fertile heterozygous for two pairs of male-sterility genes. In the progeny of the backcross C337–1–2 × C324–3, the observed ratio 17:14 is nearer 1:1 ($X^2=0.29$, $P=0.59$) than 3:1 ($X^2=6.7$, $P=0.01$), but in the other backcrosses mentioned above, with the same male-fertile parent C324–3, the ratios (25:6 and 38:6) were nearer 3:1 than 1:1, so that C324–3 was probably MS_1 ms_1 MS_2 ms_2. In the backcross progeny of C337–1–2 × C328–1, the observed ratio was 41:21, which is nearer 3:1 ($X^2=2.6$, $P=0.1$) than 1:1 ($X^2=6.5$, $P=0.01$). The F_1 male-fertile parent, C328–1, gave in F_2, 36 male-fertile and 3 male-sterile plants, or 12:1, so that it is uncertain whether this F_1 was MS_1 ms_1 ms_2 ms_2 or Ms_1 ms_1 Ms_2 ms_2. The backcross family, C218–8–1–4 × C327–3 contained 118 male-fertile and 21 male-sterile plants, a ratio considerably nearer 7:1 ($X^2=0.86$, $P=0.3$) than 3:1 ($X^2=7.5$, $P=0.006$). This family was very vigorous, and the greater fruitfulness of male-fertile as compared with male-sterile plants was especially conspicuous. Two male-fertile plants C342–1 and C342–2 from this family gave on selfing 59:5 and 31:1 ratios. The 7:1 backcross ratio suggests that the male-fertile parent C327–3 was heterozygous for three pairs of male-sterility genes. This plant itself originated in a backcross of C218–8–1–4–1 and C322–2 that consisted of 31 male-fertile and 6 male-sterile plants. Possibly a third male-sterility gene was introduced in the unrelated male-fertile parent of the F_1 plant C322–2. The total ratio in the backcross progenies, excepting C218–8–1–4 × C327–3, is 157:53 and is almost exactly the expected dihybrid ratio.

The F_2 and backcross data therefore indicate that male sterility is genetic and depends on the presence of two or, in some genotypes, possibly three recessive genes which are either independent or not closely linked.

TABLE 2.—*Relation between male sterility and other genes* [1]

Parents	Derivation	Genes entering F₁ together with male sterility	Male fertile or sterile	Progeny																	
				Chromosome I						Chromosome II		Chromosome III		Chromosome IV				Chromosome V		Chromosome VI	
				D	*d*	*P*	*p*	*S*	*s*	*R*	*r*	*Y*	*y*	*C*	*c*	*Sp*	*sp*	*A*	*a*	*L*	*l*
				No.	*No.*	*No*	*No*	*No*	*No*	*No*	*No*	*No*	*No.*	*No*	*No*	*No.*	*No*	*No*	*No.*	*No.*	*No*
C307-3	*S*	*drC*	+	76	26					75	27			79	23						
			O	14	2					11	5			14	2						
C322-2	*S*	*dS*	+	53	15			59	9												
			O	1	0			0	1												
C324-1	*S*	*PSR*	+			43	19	52	12	45	14										
			O			4	2	3	3	3	3										
C324-2	*S*	*PSRYa*	+			25	5	26	4	23	7	19	11					26	7		
			O			2	0	2	0	0	2	2	0					2	0		
C337-1	*S*	*RYCAL*	+							137	43	136	44	128	50			138	41	134	44
			O							9	4	12	1	11	4			13	2	10	5
C339-1	*S*	*dCSp*	+	193	66									178	77	165	80				
			O	13	2									10	5	11	4				
C328-1	*S*	*PSRa*	+			24	11	30	6	23	13							28	8		
			O			3	0	3	0	2	1							1	2		
C342-1	*S*	*a*	+															39	16		
			O															5	0		
C342-2	*S*	*S*	+					31	2												
			O					0	1												
C338-1	*S*	*drCSp*	+	75	16					70	18			70	31	70	32				
			O	15	4					11	7			16	3	15	3				
C218-8-1-4-1×C322-2	*B*	*d*	+	19	12																
			O	2	4																
C218-8-1-4×C327-3	*B*	*a*	+															55	62		
			O															10	12		
C337-1-2×C324-3	*B*	*RY*	+							15	1	7	9								
			O							12	2	9	5								
C337-1-7×C324-3	*B*	*R*	+							10	14										
			O							2	2										
C337-1-4×C324-3	*B*	*R*	+							18	21										
			O							2	3										

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[1] The phenotypes are represented by their corresponding gene symbols + signifies male fertile, *O*, male sterile, *S*, that progeny was derived from selfing; *B*, that it was derived from backcrossing

The percentage of seeds that germinated and were eventually recorded as plants with respect to their pollen is shown in the last column of table 1. This percentage was used as a measure of viability; the mean value was determined in families having an excess of male steriles over the expectation and in progenies with a deficiency of male steriles. The mean viability of excess male-sterile families was 64 percent and that of deficiency families 71 percent; the difference is probably not significant. Other evidence that male-sterile plants have normal viability is the fact that no consistent difference in vigor is evident between male-fertile and male-sterile plants in the field.

To test the possible influence of pollen competition, or certation, on the proportion of male-sterile progeny, an amount of pollen was applied which seemed barely sufficient to fertilize all the eggs. A few pollen grains of plants heterozygous for male sterility were picked up on a human hair and applied to the stigma of a male sterile. The average number of seeds in four fruits from limited pollination was 10, as compared with over 50 in seven fruits of the same plants from unlimited pollination. Presumably the amount of pollen was sufficiently limited to prevent certation between pollen carrying male sterility and that carrying male fertility. In one cross there were fewer male steriles from limited pollination, but in the other the relation was reversed. This suggests that pollen certation does not greatly influence the frequency of transmission of male sterility.

Alleles of certain genes present in the male-sterile parents were introduced to test the relation between the male-sterility genes and genes in six different linkage groups (tables 2 and 3).

TABLE 3.—*χ-square test of association between male sterility and other genes in the combined F_2 and backcross (B) progenies* [1]

Generation	Gene entering F_1 together with male sterility	Chromosome No.	Observed phenotypic ratios				χ^2	P	
			Male fertile		Male sterile			Positive	Negative
			X	*x*	*X*	*x*			
F_2	d_1	I	397	123	43	8	1.25		0 27
B	d_1	I	19	12	2	4	.65	0 42	
F_2	*P*	I	92	45	9	2	.45	50	
F_2	*S*	I	198	33	8	5	3.8		.05
F_2	*r*	II	145	45	22	12	1 5	.22	
F_2	*R*	II	231	77	14	10	2 4		12
B	*R*	II	43	36	16	7	1 2	.27	
F_2	*Y*	III	155	55	14	1	1 9	17	
B	*Y*	III	7	9	9	5	.58	.45	
F_2	*C*	IV	455	181	51	14	1 1	29	
F_2	*Sp*	IV	235	112	26	7	1.2	.27	
F_2	a_1	V	93	31	8	2	.00	1 0	
F_2	A_1	V	138	41	13	2	.27	60	
B	a_1	V	55	62	10	12	.01		.92
F_2	*L*	VI	134	44	10	5	.01		.92

[1] An association, measured by *P*, of male sterility with the gene entering F_1 together with male sterility is termed positive and would be evidence of genetic linkage. An association of male sterility with a gene not entering F_1 together with male sterility is negative. *X* represents the dominant, *x* the recessive phenotype. *P* is the probability that the observed frequencies would occur by chance, in case of independence. *B*=backcrossed.

The χ-square test of association, with Yates' correction, was applied.[2] As is evident from table 3, there was no significant association of male sterility with the chromosome I genes d_1 and *P*; and the apparent association with *S* is not due to linkage. There is no evident association of male sterility with *R*, *r*, *Y*, A_1, a_1, or *L* but possibly a suggestion of linkage with the genes *C* and *Sp* in chromosome IV, the odds being about 2.6 to 1 in favor of association. Clearly it is not proved that male sterility is independent of these six linkage groups but only that it is probably not closely linked with any of the genes involved in the tests. For the genetic study of male sterility alone it would probably have been better to introduce as few genes as possible allelic to those in the male-sterile parents.

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[2] FISHER, R. A. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 6, rev. and enl., 339 pp., illus. Edinburgh and London. 1936.

DISCUSSION

Male sterility in the tomato was reported by Crane.[3] It behaved as a recessive character and was believed to depend on a mutant gene. In maize 20 genetically distinct types of male sterility have been described by Emerson, Beadle, and Fraser,[4] most of them depending on single recessive genes. The dependence of male sterility on at least two genes, as in the present instance, seems to be rather exceptional. If two recessive alleles must be present to produce male sterility, they appear to be complementary genes, and male sterility should occur in F_2 from crossing male-fertile races having different male-sterility genes. Male sterility is not due to gametophytic lethals, since heterozygous plants may be quite male fertile and the backcross male sterile × (F_1 male sterile × male fertile) gave in part male-sterile progeny, whereas male sterile × unrelated male fertile gave only male-fertile progeny. It is due to genes in the sporophyte which cause the development of the microspores to be arrested and which therefore have a lethal effect.

In the tomato, fruitfulness as a rule depends on self-pollination. In the greenhouse, with many varieties hand pollination is necessary. In the field, agitation by wind is usually sufficient to shake onto the stigma the pollen that is shed within the tube formed by the anthers Bumblebees and other insects also assist to a much less extent in pollination. Since self-pollination is the rule, defective pollen is potentially a major cause of unfruitfulness. Unfruitfulness in triploid tomatoes has been shown to be due to this cause. In the present instance, since the amount of pollen produced is nearly normal, some other cause of unfruitfulness might easily be expected. One of the five unrelated male-fertile plants used in crossing is apparently heterozygous for both male-sterility genes. It is by no means improbable that some of the unfruitful plants that occur in commercial varieties are genetically male sterile. Plants heterozygous for two male-sterility genes would give on selfing only one-sixteenth of male-sterile progeny, and owing to the complete recessiveness of male sterility, they would not tend to be eliminated by mass selection. Male sterility depending on two recessive genes could be eliminated from a seed stock by single-plant selection, if a sufficiently large population from selfing of each selected parent were tested. According to Warwick,[5] on a 15:1 basis the odds are 21:1 in favor of the occurrence of at least one recessive in a population of 48 plants.

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[3] CRANE, M. B. HEREDITY OF TYPES OF INFLORESCENCE AND FRUITS IN TOMATO. Jour. Genetics 5. 1–11, illus. 1915.

[4] EMERSON, R. A., BEADLE, G. W., and FRASER, A. C. A SUMMARY OF LINKAGE STUDIES IN MAIZE N. Y. (Cornell) Agr. Expt. Sta. Mem. 180, 83 pp. 1935.

[5] WARWICK, B. L. PROBABILITY TABLES FOR MENDELIAN RATIOS WITH SMALL NUMBERS. Tex. Agr. Expt Sta. Bull. 463, 28 pp., illus. 1932. See table 4.

It is not suggested that fruitfulness in tomatoes is determined by only two or even three pairs of genes. Variation in fruitfulness occurred in these cultures which could not be related to male sterility. For example, in certain populations in which segregation of D_1 (standard) and d_1 (dwarf) occurred, the unfruitful condition was less extreme in standard plants, and where segregation of L (normal green) and l (lutescent) occurred, the normal green plants tended to be more fruitful. Evidently other genes affecting fruitfulness occur, some of which might be studied in relation to known genes.

Apparently the mutation that resulted in the formation of a gamete carrying male sterility occurred in a somatic cell or a spore mother cell of the triploid × diploid F_1 plant 30.059 .41, since only one of the three F_2 plants tested proved to be heterozygous for both male-sterility genes. Possibly the fact that the F_1 plant was a double trisomic made conditions favorable for the simultaneous occurrence of two gene mutations, but a more probable hypothesis is that this plant was already heterozygous for one mutant gene and that a second mutation occurred in it.

It is suggested that some of the unfruitful plants that occur in commercial varieties are male-sterile segregates from the selfing of fruitful and apparently normal heterozygous plants.

SUMMARY

Partially unfruitful plants occurred in an F_3 population from a simple trisomic F_2 plant having an extra H (IV) chromosome. The F_1 plant was probably a double trisomic having an extra H (IV) chromosome and another extra chromosome not identified. The pollen produced by the male sterile is nearly normal in amount, but its development is arrested. In mature anthers the pollen is distinguished in aqueous iodine by its slightly smaller size, darker color, and coarser texture and by the prevalence of starch granules in the cytoplasm. A small proportion, perhaps 2 to 3 percent, of fully developed grains occurs, but apparently they do not function. Male sterility is completely recessive and depends on at least two recessive genes, ms_1 and ms_2, both of which are necessary for male sterility. In F_2 from male sterile × male fertile, approximations to 15:1 and 3:1 ratios, and in backcrosses usually 3:1 ratios occurred. An apparent 7:1 ratio in one backcross suggests that the parents may have differed in a third pair of male-sterility genes, the third dominant allele being introduced by the male-fertile parent. One of the five unrelated male-fertile races was heterozygous for one or probably both mutant genes.

THE CALCIUM-PHOSPHORUS RATIO OF THE SKINS OF CANNING PEAS AND ITS RELATION TO MATURITY[1]

By DONALD W. BOLIN, *assistant chemist, Idaho Agricultural Experiment Station,* and WILBUR SCHROEDER, *graduate student, College of Agriculture, University of Idaho*[2]

INTRODUCTION

The chemical changes that take place in peas (*Pisum sativum* L.) with maturity have been studied by many investigators (*2, 4, 5, 8, 10, 11, 12, 13*)[3] in an effort to discover and perfect chemical methods for determining the quality of the canned product. Sayre, Willaman, and Kertesz (*11*) found that the calcium content of pea skins increases with maturity, and later, Kertesz (*9*) reported that the alcohol-insoluble solids also increased with maturity and serve as a good index to quality. Thompson and Peterson (*14*), in studying the chemical composition of the whole pea, found that calcium and phosphorus increased with maturity when the percentage was calculated on the wet basis, but remained approximately constant when the percentage of each was calculated on the oven-dry basis. Previous work at the Idaho station (*1*) has shown that the skins of mature peas have a wide calcium-phosphorus ratio.

The work described in the present paper was undertaken to obtain data on the calcium-phosphorus ratio of the raw canning-pea skins at different stages of maturity and to determine, if possible, its relationship to the quality of the canned pea.

MATERIAL AND METHODS

The raw canning peas used for this work were obtained from a commercial cannery at Ellensburg, Wash. When the peas arrived from the viner they were separated according to the standard sieve sizes and the skins were immediately removed from the cotyledons by hand. The skins from the respective sieve sizes were washed, dried, and sent to the laboratory for chemical analysis of calcium and phosphorus. From the same lot of peas, canned samples of the respective sieve sizes were taken for the organoleptic grade and for calcium and phosphorus analyses of the skins. The organoleptic grade was determined by two officials of a commercial cannery, each grading independently of the other, and their results were averaged. Where there is no organoleptic grade indicated, the canned samples were lost in transportation.

Two grams of air-dried pea skins were ashed in a platinum dish at 500° C. The ash was dissolved in 2 ml of 6 N hydrochloric acid and made up to a definite volume of 100 ml with distilled water. An aliquot of this solution, representing 0.1 g of pea skins, was analyzed

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[1] Received for publication July 19, 1938. Research Paper No. 171 of the Idaho Agricultural Experiment Station.

[2] The authors wish to express their appreciation to the Bozeman Canning Co. of Mount Vernon, Wash., for supplying the pea samples.

[3] Italic numbers in parentheses refer to Literature Cited, p. 635.

colorimetrically for phosphorus according to the method of Fiske and Subbarow (*7*).

The calcium was also determined colorimetrically, as described by Emmert (*6*). A 25-ml aliquot, corresponding to 0.5 g of the pea skins, was transferred to a 50-ml volumetric flask, made alkaline to Phenolphthalein with 10-percent sodium hydroxide and 2 ml of 10-percent sodium hydroxide added in excess. The contents were made up to volume and filtered. An aliquot of the alkaline filtrate equivalent to 0.2 g of the pea skins was transferred to a 50-ml volumetric flask, acidified with dilute sulphuric acid, and the phosphorus was determined colorimetrically as the alkaline-soluble phosphorus. Known amounts of phosphorus were added to those canned samples that did not have enough phosphorus present to precipitate all the calcium and did not give a color of sufficiently high intensity for colorimetric reading. The difference in percent between the acid-soluble phosphorus and the alkaline-soluble phosphorus was multiplied by 1.93 to obtain the percentage of calcium.

Although the colorimetric determination of calcium gave slightly higher results than the commonly used oxalate method, it offered a simple and rapid calcium determination with precise comparative results. The numerical calcium-phosphorus ratio, as given in the following tables, was derived as follows:

$$\text{Calcium-phosphorus ratio} = \frac{\text{Percent calcium} \times 100}{\text{Percent phosphorus}}$$

RESULTS AND DISCUSSION

Table 1 shows the variation in percentage of calcium and phosphorus and the calcium-phosphorus ratio of the skins obtained from different sieve sizes of two varieties of raw canning peas. The organoleptic grade is also given for a few samples.

With few exceptions the results in table 1 show an increase of calcium and a decrease of phosphorus in the raw pea skins with advancing stages of maturity, when maturity is based on seive size. The increasing calcium content and the decreasing phosphorus content of the skins result in a wider ratio as the pea matures. The increase in calcium content with maturity is in agreement with the work of Sayre, Willaman, and Kertesz (*12*), who state that there is a migration of calcium to the skin as the pea matures. Thompson and Peterson (*14*) state that no difference was found between the calcium and phosphorus content of the whole pea with maturity when calculated on an oven-dry basis; one may assume, therefore, that there is a migration of calcium from the cotyledon with advancing maturity. Boswell (*3*) reports a decrease in nonprotein nitrogen and an increase in protein nitrogen with maturity. It is possible that the migrating phosphorus from the skins combines with the increased protein nitrogen of the cotyledon as a phosphoprotein.

It is recognized that the size of the pea is not always an index to maturity. Environmental factors, such as prolonged drought, insects, etc., may sometimes prevent the enlargement of the pea without hindering maturation. In spite of these factors, however, the size of peas from the same lot, grown and secured under the same conditions, can be used as a general index to maturity.

TABLE 1.—*Calcium and phosphorus content, calcium-phosphorus ratio, and organoleptic grade of the skins of different sieve sizes of 2 varieties of raw canning peas*

Sample	Variety	Phosphorus in sieve size—			Calcium in sieve size—			Ca:P ratio $\times 100$ for sieve size—			Organoleptic grade of sieve size—		
		3	4	5	3	4	5	3	4	5	3	4	5
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*						
B	Perfection	0.376	0.329	0.263	0.215	0.265	0.302	57.2	80.5	114.8			
D		334	297	.192	240	290	292	71.9	97.6	152.1			
F		.245	214	150	214	286	264	87.3	133.6	176.0	Extra Standard	Extra Standard	Standard.
G		.400	.278	.191	312	315	338	78.0	113.3	177.0	Fancy	do	Do
A	Lot 100[1]	.393	.423	352	236	.248	248	60.1	58.6	70.5			
C		.566	432	275	196	216	278	34	50.0	101.1			
E		.222	.198	149	194	263	254	87.4	132.8	170.5			
H		258	178	.168	294	322	321	114.0	180.9	191.1	Extra standard	Standard	Standard
I		.374	.267	.221	.241	366	301	64.4	137.1	136.2	Fancy	Extra standard	Do
J		.301	.303	.209	283	338	295	94.0	111.6	140.2	Extra standard	do	Do.

[1] A selection from Profusion, developed at the Idaho station.

The above factors must be taken into consideration in interpreting the data in table 1, especially in comparing the calcium-phosphorus ratio of peas grown under different field conditions. For example, in table 1, the calcium-phosphorus ratio of sieve size 4 in sample B is 80.5, the calcium-phosphorus ratio of sieve size 3 in sample F is 87.3, indicating that sieve size 3 of sample F is more mature than sieve size 4 of sample B. This finding is further substantiated by the calcium-phosphorus ratio of sieve sizes 4 and 5 of sample F. In those samples having an organoleptic grade, there exists a fairly high degree of correlation between the calcium-phosphorus ratio and the given grade. In sample H the peas of sieve size 3 are more mature than those of sieve size 5 in samples A and C. The organoleptic grade of sample H also indicates a more mature sample of peas.

TABLE 2.—*Calcium and phosphorus content and calcium-phosphorus ratio of the skins of different sieve sizes of 2 varieties of canned peas*

[Lots and sieve sizes correspond to those of table 1]

Sample	Variety	Phosphorus in sieve size—			Calcium in sieve size—			Ca/P ratio$^{\times 100}$ for sieve size—		
		3	4	5	3	4	5	3	4	5
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*			
B	Perfection	0.179	0.172	0.150	--------	0.705	0.946	-------	409.9	630.7
C	Lot 100	196	.176	.146	0.678	.620	.715	345.9	352.3	489.7
D	Perfection	145	.130	.129	.579	.663	.670	399.3	510.0	519.4
E	Lot 100	.180	.145	.106	.599	.595	.704	332.8	410.3	664.2
H	do	.124	101	.086	.542	.526	.573	437.1	520.8	666.3
I	do	.184	.148	117	.477	595	542	259.2	402.0	463.2
J	do	.156	.137	.103	537	.546	.597	344.2	398.5	579.6

Table 2 shows the calcium and phosphorus analyses of the pea skins of the canned product from the same lots shown in table 1 with corresponding numbers and sieve sizes. The percentage of calcium in the pea skin is increased by the canning process. This agrees with the finding of Sayre, Willaman, and Kertesz, who showed an increase in the calcium content and a decrease in the ash content of the canned product. The phosphorus content of the pea skin decreases with canning. This loss of phosphorus may be accounted for in the liquor, but the reason for the increase of calcium in the skins of the canned product over that in the peas is not clear.

An increase in the calcium-phosphorus ratio of the more mature pea over that of the less mature pea is shown in table 2, but whether the calcium-phosphorus ratio in the canned pea is as good an index of maturity as it is in the raw pea is questionable because of the loss of phosphorus in the liquor and the increase of calcium in the skins of the canned product.

The results in table 3 show that the calcium-phosphorus ratio of the mature pea skins is much higher than the calcium-phosphorus ratio of the skins of either the raw canning peas or the canned product. In table 2 the highest calcium-phosphorus ratio shown for the canned product is 666.3, which is much lower than any of the ratios from the skins of the mature peas shown in table 3. In view of these wide differences, the calcium-phosphorus ratio may serve as a means of distinguishing the raw canned peas from the soaker canned peas.[4]

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[4] Soaker canned peas are mature seed peas that have been presoaked and canned.

TABLE 3.—*Calcium and phosphorus content and calcium-phosphorus ratio of the skins of mature seed peas*[1]

Variety	Calcium	Phosphorus	$Ca/P \times 100$
	Percent	*Percent*	
Blue Bell	1.00	0.089	1,124
Alaska	1.18	.084	1,405
Kaiser	1.18	.106	1,113
White Canada	1.04	.083	1,253
Earliest of All	.97	.073	1,329

[1] Except for the variety Earliest of All, the data in this table were taken from an Idaho station bulletin (*1*) and recalculated to the elemental calcium and phosphorus.

Since the increase in calcium content and the decrease in phosphorus content of the pea skins vary inversely with maturity, the ratio of these two elements should provide a good method for the determination of maturity and quality of the raw peas for canning purposes. The use of the calcium-phosphorus ratio eliminates errors arising from variations in the moisture content of the pea.

However, several comparisons must be made with well-known tests for quality before a definite calcium-phosphorus ratio can be used as an index to quality.

SUMMARY

Data have been presented which show that there is a marked variation in the calcium and phosphorus content of the pea skin at different stages of maturity. Analyses of the skins show that the calcium content and the calcium-phosphorus ratio increase and the phosphorus content decreases with maturity.

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THE RELATION OF NUTRIENTS TO TOXICITY OF ARSENIC, BORAX, AND CHLORATE IN SOILS[1]

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INTRODUCTION

Much of the knowledge of chemical herbicides is empirical. Field practices in weed control are not adequately related to basic principles (*5*)[3] and consequently are unreliable or erratic. Proper interpretation of the abundant observational data on hand awaits the development of fundamental knowledge broad enough to encompass the wide range of variables in the field environment.

Essential to this knowledge is an understanding of the relative importance of the several edaphic factors most directly involved in toxicity relations. With herbicides that act through the soil, certainly the textural grade, water-holding capacity, and crop-producing power (fertility) are of paramount importance.

Three recent papers (*6*, *9*, *14*) explain the relation of textural grade and colloid content of soils to arsenic fixation and toxicity[4] and the effect of fertility upon chlorate toxicity. Briefly, heavy soils fix large amounts of arsenic, rendering it unavailable to plants. Arsenic toxicity is high in light soils, especially in those low in colloids. The reddish color of certain soils indicates the presence of compounds high in fixing power and capable of rendering arsenic unavailable (*9*, *14*). Chlorate toxicity in soils is inversely related to fertility, being high in infertile soils and low in those that produce heavy crops (*6*).

The extreme expression of these responses selected from a group of 80 California soils is illustrated in figure 1. Here are shown a chlorate (*A*) and an arsenic (*B*) toxicity series in Aiken clay loam. This soil is low in fertility and gives a definite response to application of 5 p. p. m. of sodium chlorate. On the other hand, it is fairly heavy, high in colloids, and red in color, and consequently arsenic toxicity is low. Arsenic toxicity, as related to colloid content and certain iron

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[2] The writer is indebted to R. S. Rosenfels, assistant physiologist, Division of Cereal Crops and Diseases, for the analytical work in connection with the xylem exudates reported in the latter part of the paper, and to Jack Matley, agent, Division of Cereal Crops and Diseases, for carrying out the greenhouse culture work in the many tests reported.

[3] Italic numbers in parentheses refer to Literature Cited, p. 671.

[4] The term "toxicity" has acquired a wide variety of meanings. For the present paper the criterion adopted is the application of chemical causing an almost complete suppression of growth. This use of the word has developed in the practice of soil sterilization because the practical object is to eliminate plants. In the interpretation of toxicity curves such as those of figures 3, 5, 7, 8, 9, 10, 11, and 12, the manner in which the individual graphs approach the base line is the basis used for comparison. Though the relations in the intermediate range may have theoretic interest, only the concept of toxicity that approaches in meaning the term "minimum lethal dose" has practical significance in weed control.

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compounds, depends upon soil composition and cannot be readily altered. Fertility is the resultant of a number of factors, and chlorate toxicity might be considerably influenced if the particular ones concerned were known and understood.

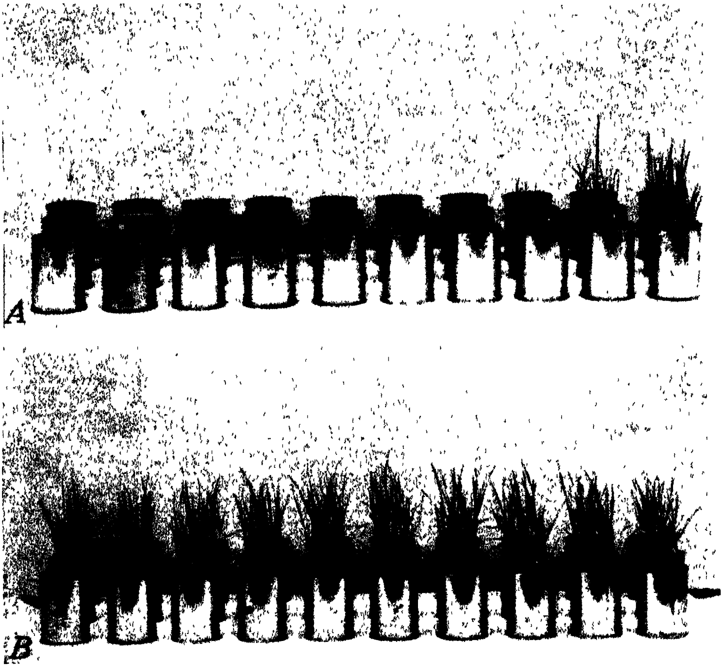

FIGURE 1.—Toxicity of sodium chlorate (*A*) and sodium arsenite (*B*) in Aiken clay loam, as shown by growth of Kanota oats. Checks are on the right, with concentrations increasing toward the left. The concentrations expressed in terms of $NaClO_3$ and As_2O_3 in air-dry soil are, from right to left, 0, 5, 15, 40, 80, 140, 220, 340, 490, and 680 p. p. m.

METHODS

Study of the plants growing in the cultures used in the experiments that have been described (*6*, *9*, *14*) indicated that those in infertile soils suffered definite mineral deficiencies. Since, in the chlorate experiments, there was a correlation between fertility and toxicity, similar greenhouse studies were begun with arsenic, borax, and chlorate in soils and in water cultures having four different levels of mineral nutrition. The nutrient levels were obtained by moistening the soil with salt solutions containing the principal nutrient elements in the proportions used by Hoagland in his culture solutions (*11*, *12*).

Table 1 summarizes descriptive data for the soils used. All samples were taken within 4 inches of the surface and, wherever possible, from fence lines where recent cultural operations had not affected the physical or chemical status of the soil.

TABLE 1.—*Name, sampling location, and moisture capacity of soils used in herbicide experiments*

No.	Type name	Sampling location (California)	Moisture [1]	Weight used
			Cubic centimeters	*Grams*
1	Fresno sandy loam	Hughson	75	600
2	Yolo fine sandy loam	Davis	85	500
3	Columbia fine sandy loam	Broderick	90	500
4	Arbuckle clay loam	Arbuckle	100	500
5	Aiken clay loam	Camino	115	400
6	Yolo adobe clay	Tracy	145	500
7	Yolo clay loam	Davis	150	500
8	Sacramento clay	Clarksburg	160	500
9	Stockton adobe clay	Stockton	160	500

[1] Volume of water required to moisten 500 g of air-dry soil to field capacity.

The method used to test toxicities consists in growing indicator plants (Kanota oats) in can cultures containing graded concentrations of the toxicant in the soil or solution used as the culture medium. Figure 1 illustrates two of these series. The data recorded are the height and fresh weight of the plants 30 days after planting.

Three toxicants were used in these tests, viz, sodium chlorate, sodium borate, and sodium arsenite. Concentrations are expressed in terms of arsenic trioxide (As_2O_3), anhydrous borax ($Na_2B_4O_7$), and sodium chlorate ($NaClO_3$), respectively.

In the chlorate cultures with complete nutrients (Hoagland's solution) added (see table 2), the nutrients were applied to the different soils in equal amounts. Concentrations are expressed as parts per million of the air-dry soil weights. For this reason the concentrations calculated on the basis of the soil moisture vary inversely with the field capacities of the soils. In the arsenic and borax cultures the nutrient elements were added in equal concentrations in the moistening solutions. In the tables, concentrations of nutrients are expressed in multiples of those that occur in Hoagland's full nutrient culture solution, and the symbol H is used to imply the nutrient concentrations that occur in a "normal" or "full" Hoagland's solution.[5] Iron was not used in the soil cultures but was included in the water cultures. The toxicants were added in the moistening solutions in like amounts in all soils, based on the air-dry weight. The concentrations run in a series as in previous experiments.

The methods so far described apply to the cultures used to test the effects of adding complete nutrient mixtures to chlorate-containing soils (table 2), arsenic-containing soils (table 3), borax-containing soils (table 4), and water cultures at different nutrient levels containing arsenic, borax, or chlorate (table 5). To find the effects of various single-salt amendments upon chlorate-containing soils, the experiments recorded in table 6 were performed. Soil cultures with the usual range of chlorate concentrations were replicated in 10 different soils in the greenhouse. The various series on each soil received the following: (1) Tap water; (2) 2H solution; (3) nitrate, 30 millimols of KNO_3 per liter; (4) nitrate, 30 millimols of $NaNO_3$ per liter; (5) nitrate, 15 millimols of $Ca(NO_3)_2$ per liter; (6) potash, 12 millimols of KNO_3 per liter; (7) potash, 12 millimols of KCl per liter; (8)

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[5] This solution contains per liter 5 cc M KNO_3, 5 cc M $Ca(NO_3)_2$, 2 cc M $MgSO_4$, and 1 cc M KH_2PO_4.

potash, 6 millimols of K_2SO_4 per liter; (9) phosphate, 2 millimols of KH_2PO_4 per liter; (10) phosphate, 2 millimols of NaH_2PO_4 per liter; (11) phosphate, 2 millimols of H_3PO_4 per liter; (12) ammonia, 30 millimols of NH_4NO_3 per liter; (13) ammonia, 12 millimols of NH_3NO_3 per liter; (14) ammonia, 30 millimols of NH_4Cl per liter; (15) ammonia, 15 millimols of $(NH_4)_2SO_4$ per liter.

Finally, parallel series of experiments were run in water cultures, with graded chlorate concentrations in various single-salt solutions (table 7). The plants were started in sand, transferred to $\frac{H}{10}$ solution, where they remained for 7 days to become established, and then shifted to the chlorate-containing culture solutions.

RESULTS OF EXPERIMENTS

TOXICITY IN SOILS TREATED WITH COMPLETE NUTRIENTS

CHLORATE

Data on the toxicity of sodium chlorate at four nutrient levels in nine soils are presented in table 2. The values given are the average height and the total fresh weight for the 10 plants grown in each can. To better illustrate these results, figure 2 shows sets of plants in Yolo sandy loam, Aiken clay loam, and Yolo adobe clay. Extreme effects of plant nutrients in lowering chlorate toxicity are shown in the Aiken soil.

TABLE 2.—*Toxicity of sodium chlorate at 4 nutrient levels in 9 California soils as shown by growth of Kanota oats*

[Plants harvested Feb. 17, 1936]

AIKEN CLAY LOAM

$NaClO_3$ in air-dry soil (p. p. m.)	Water		2.2 H		4.3 H		8.7 H	
	Height	Weight	Height	Weight	Height	Weight	Height	Weight
	Centimeters	*Gram*	*Centimeters*	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*
5	6	0.3	23	5.0	30	7.1	30	7.1
15	6	.2	25	5.6	20	4.8	28	6.9
40	3	.1	17	2.9	20	3.7	25	5.9
80	0	.0	14	2.1	18	3.4	23	4.6
140	0	.0	15	1.6	19	2.4	26	5.0
220	0	.0	8	.5	16	1.8	21	3.2
340	0	.0	5	.3	9	.7	22	2.8
490	0	.0	5	.2	5	.3	10	1.0
680	0	.0	3	.1	5	.1	8	.6
Check	8	.4	20	4.6	24	6.3	29	7.4

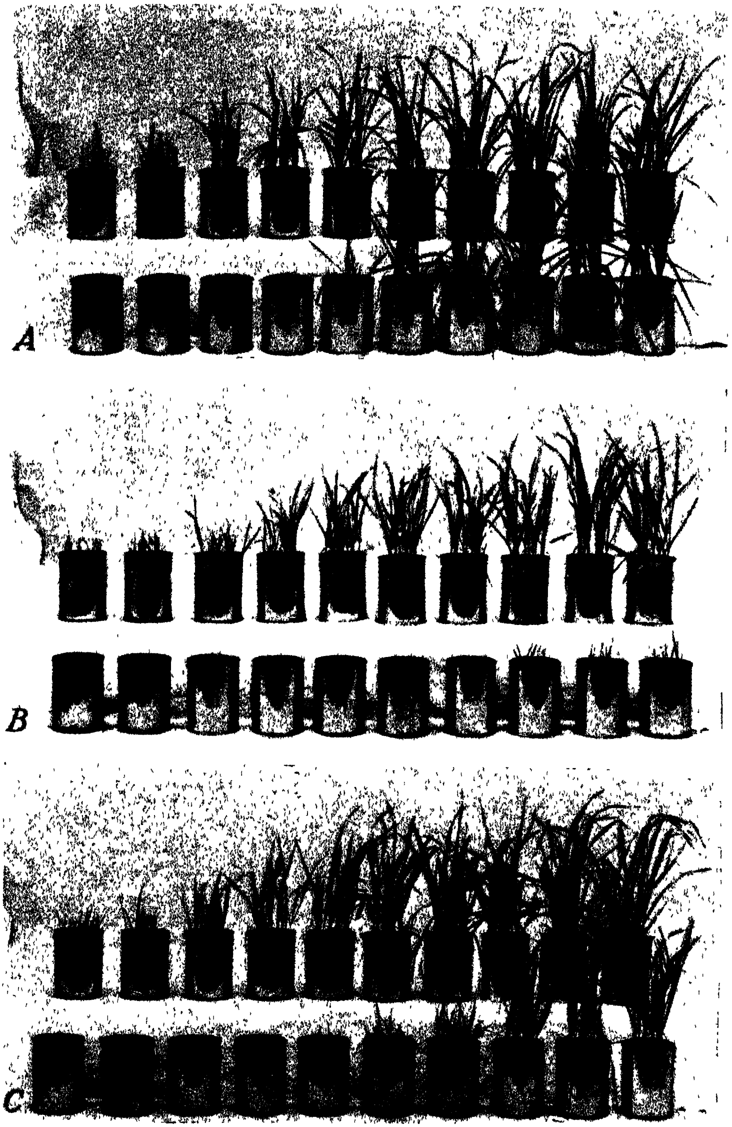

FIGURE 2.—Chlorate toxicity in three California soils in relation to nutrient level: *A*, Yolo sandy loam; *B*, Aiken clay loam; *C*, Yolo adobe clay. The lower series in each soil is the check; the checks were moistened with chlorate in tap water. The upper series received plant nutrients in the applied moistening solution approximately equivalent to a 6H solution in the Yolo sandy loam, a 4H solution in the Aiken clay loam, and a 3H solution in the Yolo adobe clay. The concentrations expressed in terms of $NaClO_3$ in air-dry soil, from right to left, are 0, 5, 15, 40, 80, 140, 220, 340, 490, and 680 p. p. m.

TABLE 2.—*Toxicity of sodium chlorate at 4 nutrient levels in 9 California soils as shown by growth of Kanota oats*—Continued

[Plants harvested Feb. 17, 1936]

ARBUCKLE CLAY LOAM

$NaClO_3$ in air-dry soil (p p m)	Water		2.5 H		5.0 H		10.0 H	
	Height	Weight	Height	Weight	Height	Weight	Height	Weight
	Centimeters	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*
5	24	4.8	33	10.4	36	13.9	38	11.1
15	29	6.6	32	10.5	36	13.0	37	10.9
40	16	.9	30	9.4	34	11.5	35	10.4
80	8	.5	28	7.1	33	9.8	33	9.4
140	5	1	23	4.5	28	6.0	31	6.6
220	4	.1	16	1.2	22	3.3	27	5.0
340	3	0	8	.6	15	1.2	23	3.2
490	0	.0	5	3	8	.4	16	1.2
680	0	.0	5	.2	5	2	13	.9
Check	25	4.4	33	10.9	34	14.3	40	13.2

STOCKTON ADOBE CLAY

$NaClO_3$ in air-dry soil (p p m)	Water		1.6 H		3.1 H		6.2 H	
	Height	Weight	Height	Weight	Height	Weight	Height	Weight
	Centimeters	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*
5	25	5.0	31	9.8	34	12.1	39	13.8
15	25	4.0	33	10.1	35	11.1	37	14.1
40	17	1.4	32	7.8	35	10.8	39	14.2
80	7	.4	28	6.6	34	5.9	39	12.6
140	6	.1	23	5.9	28	4.7	36	9.9
220	4	.1	19	1.8	22	3.0	32	6.7
340	0	.0	10	.6	17	1.2	25	3.6
490	0	.0	8	.6	10	6	24	2.9
680	0	.0	6	3	9	5	22	2.0
Check	25	5.5	30	9.5	32	12.5	40	14.9

YOLO ADOBE CLAY

$NaClO_3$ in air-dry soil (p. p. m.)	Water		1.7 H		3.4 H		6.9 H	
	Height	Weight	Height	Weight	Height	Weight	Height	Weight
	Centimeters	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*
5	28	5.8	34	10.9	87	13.1	35	12.2
15	22	3.0	31	8.9	33	12.3	37	12.5
40	15	1.2	31	8.1	31	10.4	34	9.5
80	9	.5	26	5.3	31	7.5	35	10.0
140	6	.2	24	4.0	26	5.2	33	7.5
220	4	.1	17	1.6	22	3.3	30	4.8
340	0	.0	8	.5	15	1.3	25	3.2
490	0	.0	7	.5	9	.6	17	1.4
680	0	0	4	.3	7	.3	11	.8
Check	27	5.6	34	10.3	36	13.0	37	12.4

TABLE 2.—*Toxicity of sodium chlorate at 4 nutrient levels in 9 California soils as shown by growth of Kanota oats*—Continued

[Plants harvested Feb. 17, 1936]

YOLO FINE SANDY LOAM

NaClO₃ in air-dry soil (p. p. m.)	Water		2.9 H		5.9 H		11.8 H	
	Height	Weight	Height	Weight	Height	Weight	Height	Weight
	Centimeters	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*
5	27	5.6	31	9.7	32	8.9	28	5.0
15	28	4.8	31	9.8	34	9.4	30	5.7
40	29	4.9	31	8.8	33	8.3	32	6.9
80	24	3.2	29	8.0	32	7.8	33	6.9
140	13	1.1	29	5.7	30	5.9	28	4.3
220	5	1	24	3.5	24	4.0	25	3.4
340	4	1	13	.9	19	1.7	20	2.4
490	0	0	13	5	12	1.0	16	1.5
680	0	0	5	2	11	.6	12	8
Check	28	5.6	32	9.4	30	8.5	29	4.7

FRESNO SANDY LOAM

NaClO₃ in air-dry soil (p. p. m.)	Water		3.3 H		6.7 H		13.3 H	
	Height	Weight	Height	Weight	Height	Weight	Height	Weight
	Centimeters	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*
5	32	8.4	35	10.7	32	8.3	27	4.0
15	31	8.3	34	9.0	31	6.0	26	4.0
40	30	6.0	34	8.6	32	6.0	25	3.4
80	23	3.3	31	6.9	28	5.4	22	2.9
140	18	1.8	28	5.0	26	4.3	22	2.6
220	8	5	24	3.1	21	2.9	19	2.0
340	0	0	19	1.9	17	1.5	13	1.0
490	0	0	10	7	8	.6	12	.8
680	0	0	0	.0	4	2	9	4
Check	29	7.9	36	11.8	34	9.4	28	4.7

SACRAMENTO CLAY

NaClO₃ in air-dry soil (p. p. m.)	Water		1.5 H		3.1 H		6.2 H	
	Height	Weight	Height	Weight	Height	Weight	Height	Weight
	Centimeters	*Gram*	*Centimeters*	*Gram*	*Centimeters*	*Gram*	*Centimeters*	*Gram*
5	27	9.0	32	9.5	30	8.9	31	8.7
15	27	8.4	30	9.1	30	8.3	32	8.5
40	26	7.3	28	8.0	28	7.5	31	7.9
80	25	6.3	26	7.3	28	7.2	29	7.9
140	20	4.4	26	6.1	28	7.0	28	7.3
220	14	2.1	24	4.8	26	6.0	28	6.0
340	7	.7	18	3.7	22	4.5	24	5.0
490	4	.4	12	2.6	18	3.0	22	3.6
680	3	1	10	1.2	10	1.8	19	2.4
Check	26	8.6	31	10.1	35	9.5	33	8.9

TABLE 2.—*Toxicity of sodium chlorate at 4 nutrient levels in 9 California soils as shown by growth of Kanota oats*—Continued

[Plants harvested Feb. 17, 1936]

COLUMBIA FINE SANDY LOAM

$NaClO_3$ in air-dry soil (p. p. m.)	Water		2.8 H		5.5 H		11.0 H	
	Height	Weight	Height	Weight	Height	Weight	Height	Weight
	Centimeters	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*
5	33	11.0	34	9.5	32	8.5	31	6.5
15	32	10.4	33	9.1	32	9.2	31	6.7
40	33	8.8	33	9.4	35	9.7	31	6.7
80	31	6.5	32	7.7	32	7.7	30	6.4
140	27	4.4	30	6.0	30	6.7	30	6.5
220	25	4.1	28	5.4	25	4.5	29	5.8
340	13	1.0	20	2.5	23	3.2	25	3.4
490	9	.6	19	2.1	19	2.0	21	2.3
680	5	.1	14	1.0	16	1.5	18	1.7
Check	34	11.4	33	9.8	32	9.3	30	7.1

YOLO CLAY LOAM

$NaClO_3$ in air-dry soil (p. p. m.)	Water		1.7 H		3.3 H		6.7 H	
	Height	Weight	Height	Weight	Height	Weight	Height	Weight
	Centimeters	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*
5	33	17.4	37	16.0	39	15.7	43	13.0
15	36	16.5	38	16.2	39	14.8	40	12.1
40	31	11.3	39	15.8	40	14.6	41	14.4
80	31	9.1	37	13.8	38	13.5	41	14.4
140	29	4.9	34	9.4	33	10.4	40	11.8
220	20	2.6	31	7.0	30	7.8	35	9.0
340	9	.9	25	3.3	27	3.8	30	5.5
490	6	.3	15	2.0	22	2.9	30	5.3
680	4	.1	8	.9	16	1.3	24	2.1
Check	35	17.0	38	17.5	41	15.9	38	13.5

Data from table 2 showing the relation of nutrient level to chlorate toxicity in Yolo fine sandy loam, Yolo adobe clay, and Aiken clay loam are plotted in figure 3. In Yolo adobe clay increasing the nutrient supply reduced the toxicity until at the highest level the plants survived at a chlorate concentration of 680 p. p. m. In the Aiken soil the results were similar. In the Yolo fine sandy loam the extremely high nutrient concentration at the higher levels caused some reduction in growth. Judging from the appearance of the plants, this resulted from excessive nitrates. However, at chlorate concentrations of 340 p. p. m. and over, the curves have the same relative positions as they do in the other soils. This same relation appeared with other soils tested, indicating that the higher the nutrient level in the soil the lower the chlorate toxicity, even where the nutrients are so concentrated as to reduce growth in the chlorate-free check cultures.

ARSENIC

Similar tests were carried out with arsenic and borax on seven of the same soils. To facilitate the setting up of the experiments and to aid in comparisons and analyses of the data, nutrients were added in like concentrations in the moistening solutions. Data on the arsenic tests appear in table 3, and results are illustrated in figures 4 and 5.

Although the soils and crops varied widely in these tests, convergence of the curves for all nutrient levels at the base line confirm the conclusion that arsenic toxicity is unrelated to the soil's fertility but is determined by its textural grade.

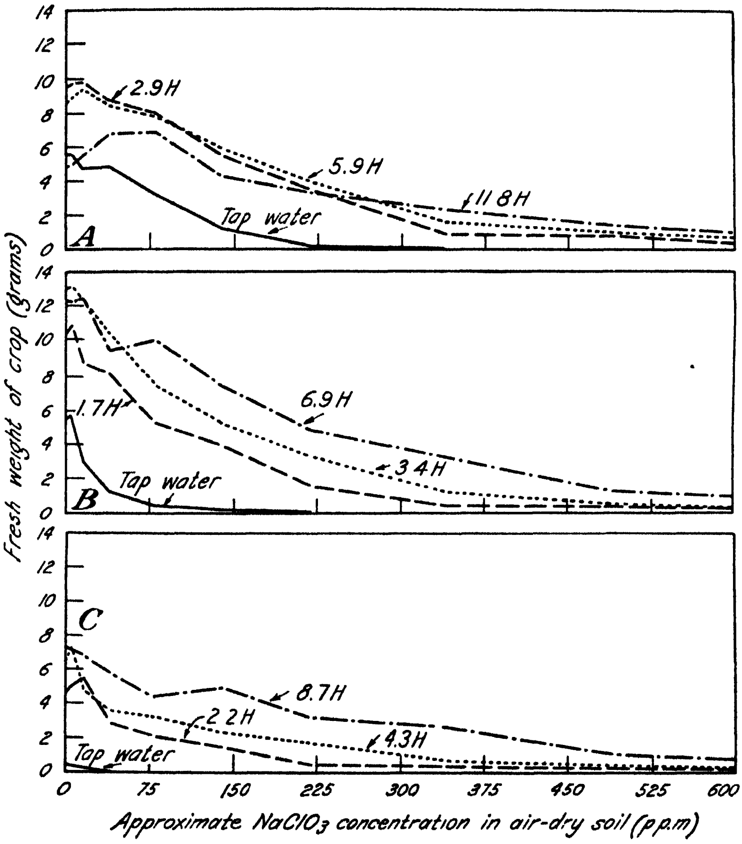

FIGURE 3.—Relation of chlorate toxicity to nutrient level in the soil: *A*, Yolo fine sandy loam; *B*, Yolo adobe clay; *C*, Aiken clay loam. The approximate nutrient level of each culture series is indicated on the individual curves, expressed, in terms of the soil solution, as multiples of concentrations used in a standard Hoagland's nutrient solution.

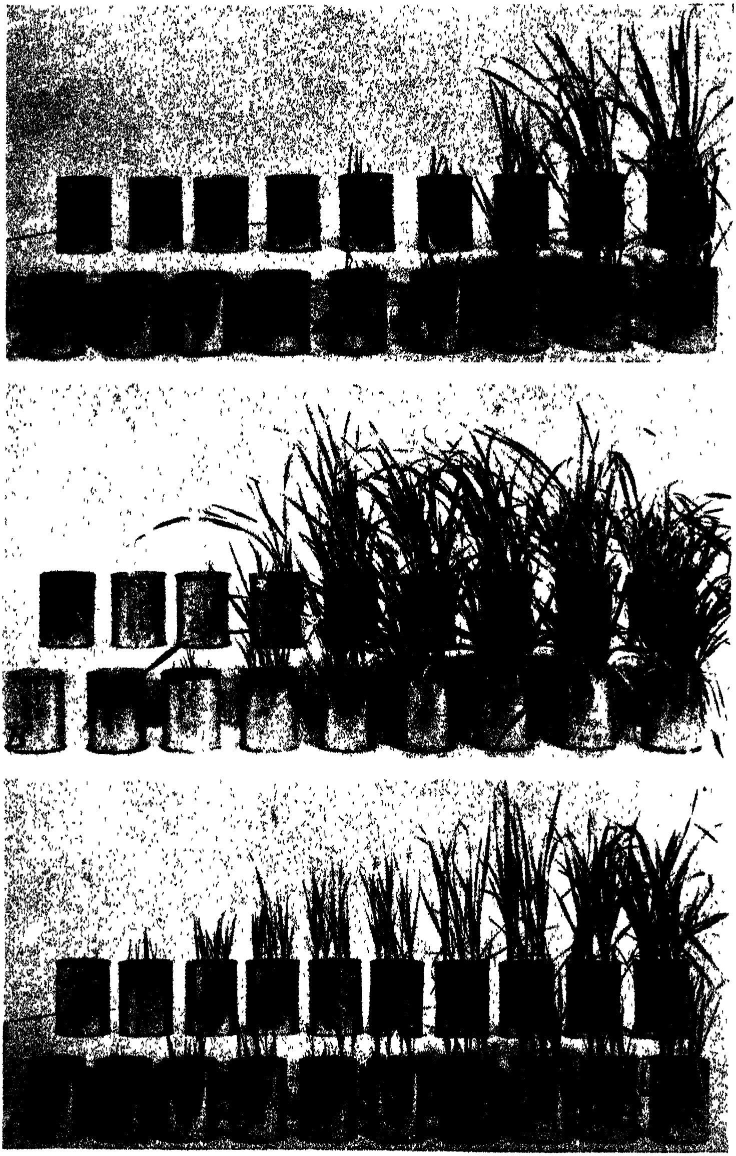

FIGURE 4.—Arsenic toxicity in three soils in relation to nutrient level: *A*, Fresno sandy loam; *B*, Yolo clay loam; *C*, Stockton adobe clay. The lower series in each soil has been moistened with sodium arsenite in tap water; the upper contains like arsenic concentrations applied in a 4H solution. The concentrations, expressed in terms of As_2O_3 in air-dry soil from right to left, are 0, 15, 40, 80, 140, 220, 340, 490, and 680 p. p. m. A 920 p. p. m. culture is included in the Stockton adobe clay.

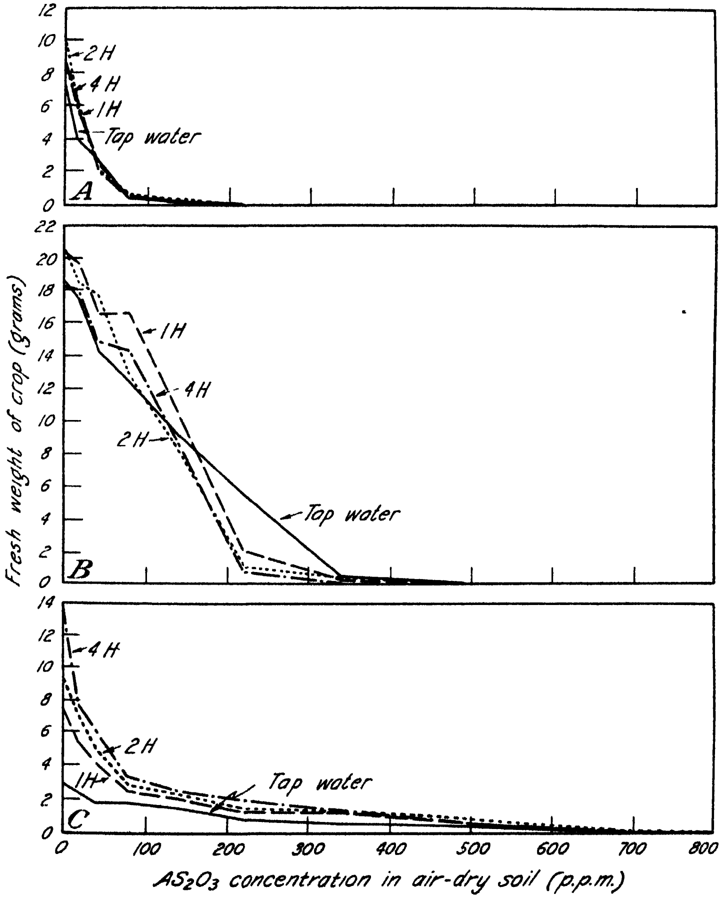

FIGURE 5.—Arsenic toxicity at four nutrient levels in three soils: *A*, Fresno sandy loam; *B*, Yolo clay loam; *C*, Stockton adobe clay.

TABLE 3.—*Toxicity of sodium arsenite at 4 nutrient levels in 7 California soils as shown by growth of Kanota oats*

[Plants harvested Apr. 24, 1937]

FRESNO SANDY LOAM

As_2O_3 in air-dry soil (p. p. m.)	Water		1 H		2 H		4 H	
	Height	Weight	Height	Weight	Height	Weight	Height	Weight
	Centimeters	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*
15	26	4.0	31	5.8	32	5.7	30	6.3
40	24	2.6	22	2.3	22	2.0	17	1.9
80	8	5	8	4	9	.6	8	.4
140	7	.2	6	2	6	.3	6	2
220	0	.0	0	0	0	.0	0	0
340	0	0	0	.0	0	.0	0	0
490	0	.0	0	0	0	.0	0	0
680	0	.0	0	.0	0	.0	0	.0
920	0	.0	0	.0	0	.0	0	0
Check	27	7.2	32	8.8	35	10.3	35	8.8

COLUMBIA FINE SANDY LOAM

As_2O_3 in air-dry soil (p. p. m.)	Water Height	Water Weight	1 H Height	1 H Weight	2 H Height	2 H Weight	4 H Height	4 H Weight
15	29	11.1	34	10.5	31	11.1	33	8.9
40	32	9.9	32	9.3	30	8.4	27	7.0
80	27	5.5	28	5.8	26	5.0	30	4.7
140	11	1.4	17	1.9	15	1.3	9	8
220	6	2	5	1	6	.3	0	0
340	0	.0	0	.0	0	0	0	0
490	0	0	0	.0	0	0	0	0
680	0	0	0	0	0	0	0	.0
920	0	0	0	.0	0	0	0	0
Check	30	13.5	33	13.3	31	12.3	31	10.6

YOLO FINE SANDY LOAM

As_2O_3 in air-dry soil (p. p. m.)	Water Height	Water Weight	1 H Height	1 H Weight	2 H Height	2 H Weight	4 H Height	4 H Weight
15	25	5.5	27	7.5	26	7.9	30	8.3
40	23	4.7	25	6.1	27	6.8	29	6.8
80	23	4.4	26	6.2	26	5.1	28	5.6
140	17	2.4	23	3.3	25	3.7	15	1.4
220	7	4	9	.6	7	.3	8	.5
340	0	0	0	0	0	.0	0	0
490	0	.0	0	0	0	.0	0	.0
680	0	0	0	.0	0	.0	0	.0
920	0	0	0	0	0	0	0	0
Check	25	5.4	26	7.4	28	8.8	33	8.9

YOLO CLAY LOAM

As_2O_3 in air-dry soil (p. p. m.)	Water Height	Water Weight	1 H Height	1 H Weight	2 H Height	2 H Weight	4 H Height	4 H Weight
15	31	17.5	33	19.6	34	18.5	36	18.0
40	28	14.2	34	16.5	37	17.8	38	14.9
80	30	12.4	35	16.6	31	12.8	33	14.2
140	33	8.9	34	10.5	20	8.1	33	8.7
220	26	5.4	10	2.0	12	1.0	7	.6
340	6	.3	6	.2	5	.3	0	.0
490	0	.0	0	.0	0	.0	0	.0
680	0	.0	0	.0	0	.0	0	.0
920	0	.0	0	0	0	.0	0	.0
Check	33	18.6	33	20.2	34	20.4	35	18.3

ARBUCKLE CLAY LOAM

As_2O_3 in air-dry soil (p. p. m.)	Water Height	Water Weight	1 H Height	1 H Weight	2 H Height	2 H Weight	4 H Height	4 H Weight
15	24	4.2	26	7.2	30	7.8	32	10.5
40	23	3.6	26	5.8	30	7.5	31	7.1
80	19	3.3	27	5.5	29	6.0	31	6.0
140	22	3.0	25	4.2	30	4.6	31	4.9
220	18	2.9	14	1.8	15	2.0	18	1.9
340	10	1.5	18	1.5	11	1.2	12	.9
490	7	.4	8	.4	7	.3	10	.6
680	0	.0	0	.0	6	.1	0	.0
920	0	.0	0	.0	0	.0	0	.0
Check	25	4.5	27	7.4	28	8.4	33	12.1

TABLE 3.—*Toxicity of sodium arsenite at 4 nutrient levels in 7 California soils as shown by growth of Kanota oats*—Continued

[Plants harvested Apr. 24, 1937]

STOCKTON ADOBE CLAY

As_2O_3 in air-dry soil (p. p. m.)	Water		1 H		2 H		4 H	
	Height	Weight	Height	Weight	Height	Weight	Height	Weight
	Centimeters	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*
15	16	2.3	24	5.5	26	7.2	26	7.3
40	16	1.8	22	4.0	22	4.8	30	6.9
80	17	1.8	20	2.5	23	2.9	27	3.2
140	16	1.5	17	2.0	18	2.3	20	2.4
220	11	.8	13	1.3	13	1.5	17	2.0
340	8	.6	11	1.3	13	1.4	13	1.4
490	7	.4	9	.7	10	1.0	9	.7
680	5	.1	5	.1	5	.2	6	.2
920	0	.0	0	.0	0	.0	0	.0
Check	17	2.9	24	7.4	24	9.2	33	14.1

AIKEN CLAY LOAM

As_2O_3 in air-dry soil (p. p. m.)	Water		1 H		2 H		4 H	
	Height	Weight	Height	Weight	Height	Weight	Height	Weight
15	23	3.5	26	4.7	25	4.8	29	5.8
40	23	3.4	26	4.0	24	4.3	26	4.7
80	21	2.9	23	3.9	27	4.2	26	4.2
140	21	2.4	23	3.7	23	3.7	24	3.6
220	21	2.2	19	3.0	22	3.0	21	2.9
340	22	2.1	19	2.4	18	2.3	20	2.5
490	17	1.8	17	2.2	16	1.8	18	2.2
680	10	1.1	14	1.8	15	1.3	11	1.2
920	8	.7	14	1.2	12	1.2	7	.7
Check	22	3.2	25	5.1	23	5.3	27	5.9

BORAX

Boron compounds, being nonpoisonous to animals, offer promise as soil sterilizers (*7*, *8*). Tests on a number of different boron salts indicate that the content of elemental boron, and not the form of compound in which it occurs, is the major factor involved in toxicity. Herbicidal use depends on solubility as well as toxicity (*8*). Borax, being readily soluble, was used in the toxicity experiments. Concentrations are expressed in terms of anhydrous borax ($Na_2B_4O_7$).

Table 4 contains the data on borax toxicity at four nutrient levels in seven soils. Results in Frenso sandy loam, Arbuckle clay loam, and Stockton adobe clay are shown in figures 6 and 7. As with arsenic, the toxicity evidently varies with textural grade, being lower in the heavy soils. Expressed as crop reduction compared with the check yield, toxicity seems unrelated to fertility. Although borax is held in soils much less firmly than arsenic against the leaching effects of moving water (*8*), the results illustrated here show that it behaves much like arsenic in its toxicity relations.

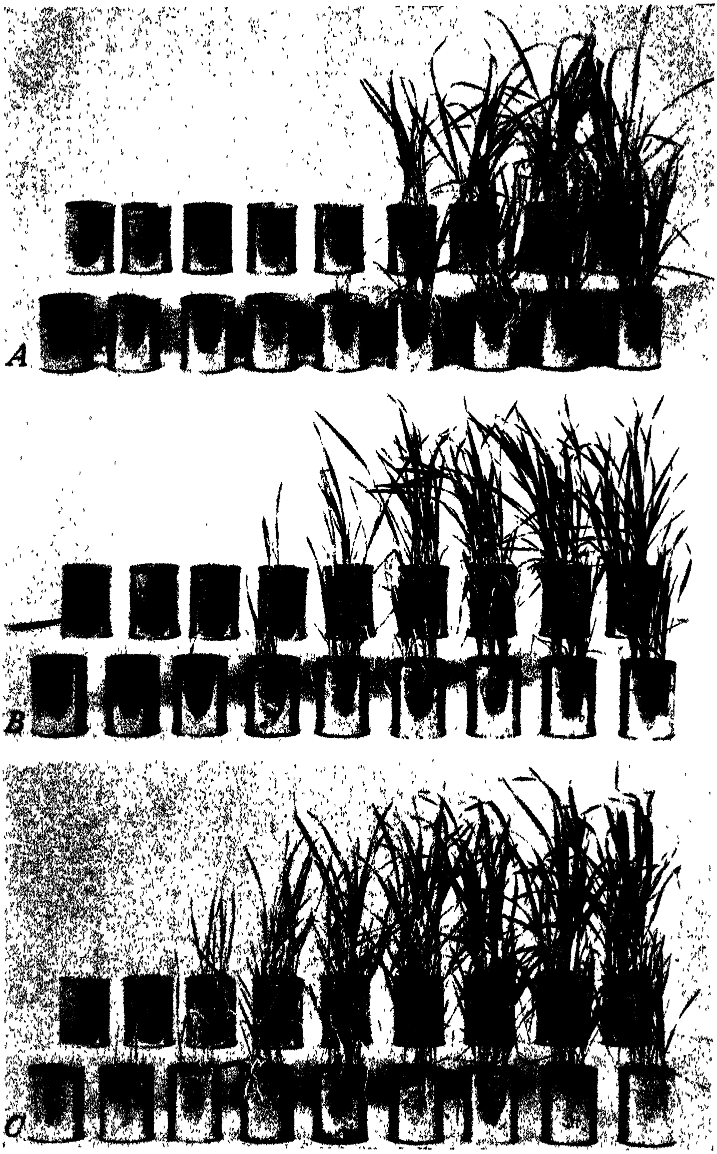

FIGURE 6.—Borax toxicity in three soils in relation to nutrient level: *A*, Fresno sandy loam; *B*, Arbuckle clay loam; *C*, Stockton adobe clay. The top row of cultures in each soil was moistened with 4H solution, the lower with tap water. The concentrations, expressed in terms of $Na_2B_4O_7$ in air-dry soil, from right to left, are 0, 15, 40, 80, 140, 220, 340, 490, and 680 p. p. m.

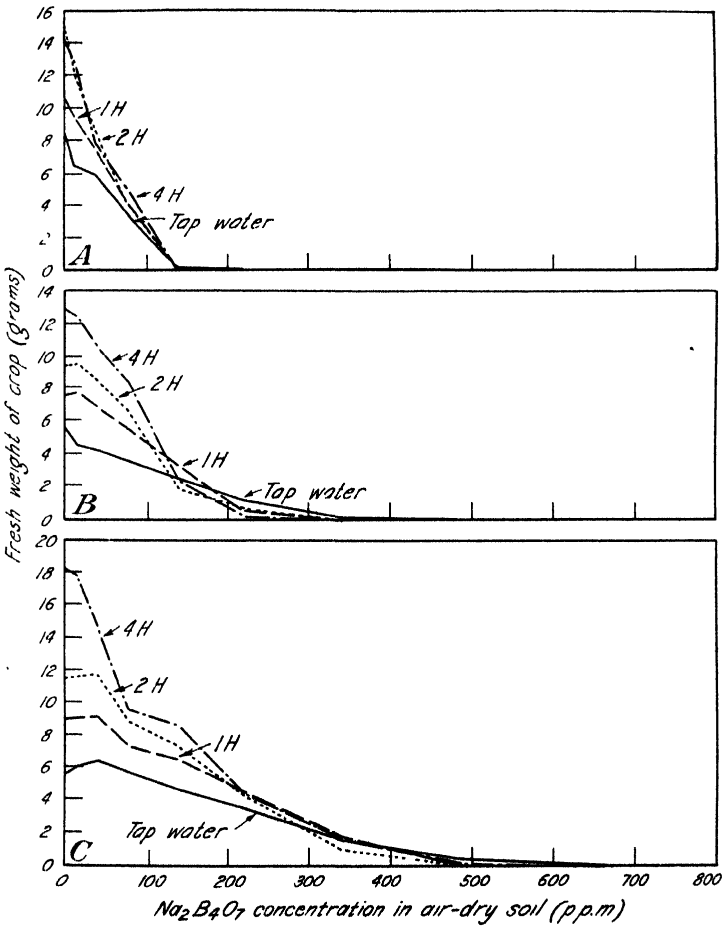

FIGURE 7.-- Borax toxicity at four nutrient levels in three soils: *A*, Fresno sandy loam; *B*, Arbuckle clay loam; *C*, Stockton adobe clay.

TABLE 4.—*Toxicity of borax at 4 nutrient levels in 7 California soils as shown by growth of Kanota oats*

[Plants harvested Apr. 22, 1936]

$Na_2B_4O_7$ in air-dry soil (p. p. m.)	Water		1 H		2 H		4 H	
	Height	Weight	Height	Weight	Height	Weight	Height	Weight
	Centimeters	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*
FRESNO SANDY LOAM								
15	34	6.5	37	9.2	42	11.6	45	12.5
40	35	5.7	37	7.4	40	8.0	37	7.2
80	23	2.6	30	3.4	33	3.2	35	3.7
140	6	.2	0	.0	0	.0	0	.0
220	0	.0	0	.0	0	0	0	.0
340	0	.0	0	.0	0	.0	0	0
490	0	.0	0	0	0	.0	0	0
680	0	.0	0	.0	0	0	0	.0
920	0	0	0	.0	0	.0	0	.0
Check	34	8.5	37	10.7	42	15.0	45	14.2
YOLO FINE SANDY LOAM								
15	27	4.9	30	8.6	33	9.8	37	7.2
40	27	4.7	31	7.2	33	8.8	34	8.8
80	27	3.0	26	2.7	27	3.5	30	4.8
140	8	.5	8	.4	7	.2	7	.2
220	0	0	0	.0	5	.1	0	.0
340	0	0	0	.0	0	.0	0	.0
490	0	0	0	.0	0	.0	0	.0
680	0	0	0	.0	0	.0	0	.0
920	0	0	0	0	0	0	0	.0
Check	27	6.7	32	9.9	33	11.2	36	9.3
COLUMBIA FINE SANDY LOAM								
15	40	13.4	40	14.3	40	16.3	45	17.1
40	39	12.8	43	13.8	43	13.6	46	12.0
80	34	7.5	36	8.7	39	11.5	41	11.1
140	18	1.2	12	1.5	20	1.8	11	1.0
220	0	.0	0	.0	0	.0	0	.0
340	0	.0	0	0	0	0	0	.0
490	0	.0	0	.0	0	0	0	.0
680	0	.0	0	0	0	.0	0	.0
920	0	0	0	.0	0	.0	0	.0
Check	40	15.0	43	16.7	45	17.7	46	16.7
ARBUCKLE CLAY LOAM								
15	27	4.5	32	7.7	32	9.5	37	12.2
40	27	4.1	31	6.8	33	8.4	36	10.4
80	27	3.4	29	5.5	32	6.5	37	8.2
140	26	2.4	27	3.2	20	1.9	26	2.3
220	15	1.1	10	.8	10	.8	5	.2
340	7	.2	0	.0	0	.0	0	.0
490	0	.0	0	.0	0	.0	0	.0
680	0	0	0	.0	0	.0	0	.0
920	0	0	0	.0	0	.0	0	.0
Check	27	5.6	30	7.5	32	9.3	35	12.8
YOLO CLAY LOAM								
15	39	18.0	38	18.7	40	19.0	42	19.6
40	38	16.4	42	14.8	42	17.2	41	16.2
80	37	13.7	40	13.8	40	14.9	43	14.9
140	37	10.1	38	10.0	37	8.1	36	7.8
220	18	1.2	8	.7	24	1.7	8	1.5
340	0	.0	0	.0	0	.0	0	.0
490	0	.0	0	.0	0	.0	0	.0
680	0	.0	0	.0	0	.0	0	.0
920	0	.0	0	.0	0	.0	0	.0
Check	37	19.5	37	20.7	40	21.2	40	22.1

TABLE 4.—*Toxicity of borax at 4 nutrient levels in 7 California soils as shown by growth of Kanota oats*—Continued

[Plants harvested Apr. 22, 1936]

AIKEN CLAY LOAM

$Na_2B_4O_7$ in air-dry soil (p. p. m.)	Water		1 H		2 H		4 H	
	Height	Weight	Height	Weight	Height	Weight	Height	Weight
	Centimeters	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*
15	24	2.8	24	3.3	26	3.9	30	5.2
40	24	2.5	24	3.3	26	3.9	30	5.0
80	22	2.2	24	3.4	25	3.6	28	4.5
140	20	1.9	23	3.0	24	2.9	24	3.4
220	19	1.7	19	2.4	18	2.2	19	2.1
340	10	1.0	10	1.3	10	1.1	9	.8
490	3	.3	5	.4	5	.3	5	.2
680	0	.0	0	.0	0	.0	0	.0
920	0	.0	0	.0	0	.0	0	.0
Check	22	2.7	24	3.3	26	3.8	31	5.6
STOCKTON ADOBE CLAY								
15	31	6.0	33	9.0	38	11.5	42	17.8
40	30	6.4	36	9.1	39	11.7	41	14.7
80	31	5.7	34	7.2	36	8.9	37	10.5
140	31	4.7	33	6.6	39	7.4	37	8.7
220	27	3.7	30	4.7	32	4.4	33	4.5
340	19	1.5	21	1.8	17	1.0	12	1.6
490	7	.5	0	.0	0	.0	6	.2
680	0	.0	0	.0	0	.0	0	.0
920	0	.0	0	.0	0	0	0	.0
Check	29	5.5	34	9.0	37	11.4	42	18.1

TOXICITY IN WATER CULTURES WITH COMPLETE NUTRIENTS

To find the effects of the soils upon the action of the chemicals under investigation, and to set a standard for comparing the various soils and chemicals, water-culture experiments were conducted at four nutrient levels and with varying concentrations of the toxicants. The results with three chemicals are reported in table 5, and these data are plotted in figure 8.

The outstanding point in these data is the extreme toxicity of arsenic. This chemical was less toxic than chlorate in some soils (for instance, Aiken clay loam, as shown in fig. 1). The soil profoundly affects arsenic toxicity (*14*), reducing it manyfold even in the coarser soils, where arsenic is highly toxic. Since arsenic toxicity is not affected by nutrient level, the solubility or availability to the plant seems chiefly concerned. To provide a lethal concentration in the soil, from 10 to 100 times the amount needed in the soil solution must be applied, depending upon the textural grade of the soil. The relation of fixation by the soil to arsenic toxicity has been discussed in a current publication (*14*).

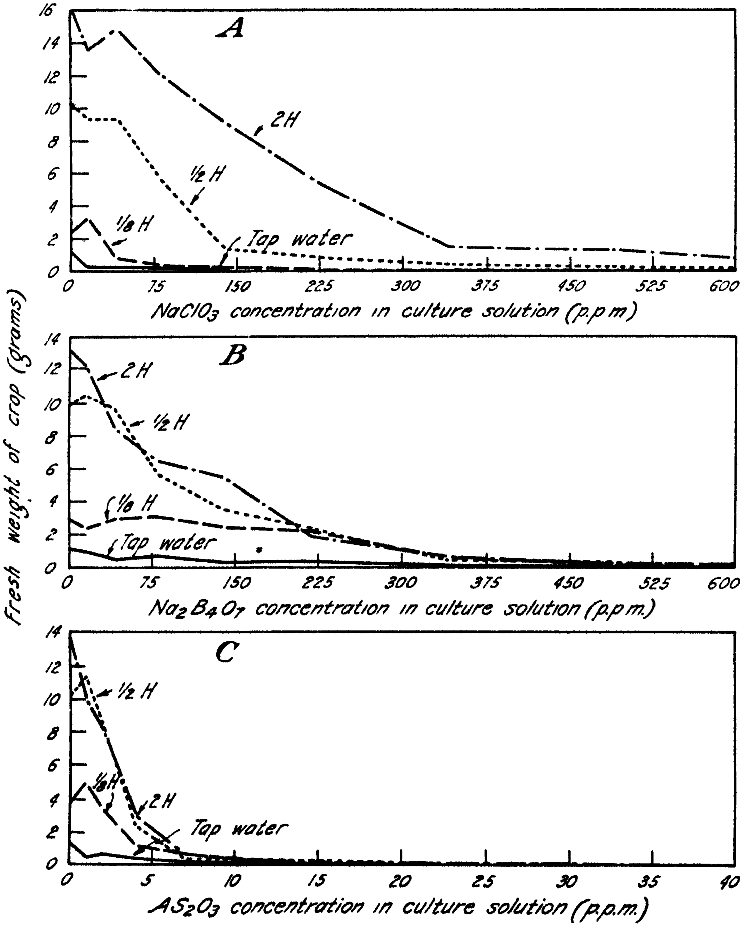

FIGURE 8.—Chlorate (*A*), borax (*B*), and arsenic (*C*) toxicity in culture solutions at four nutrient levels. Note the extremely high toxicity of arsenic, the scale being 15 times that of the other graphs.

TABLE 5.—*Toxicity of sodium chlorate, sodium arsenite, and borax in water cultures at 4 nutrient levels as shown by growth of Kanota oats*

[Plants harvested June 12, 1936]

Toxicant in culture solution (p. p. m.)	Water		⅛ H		½ H		2 H	
	Height	Weight	Height	Weight	Height	Weight	Height	Weight
	Centimeters	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*	*Centimeters*	*Grams*
SODIUM CHLORATE								
15	8	0.2	17	3.3	20	9.4	37	13.7
40	7	2	8	9	27	9.4	38	14.9
80	7	.2	8	.5	25	5.8	38	12.1
140	6	.1	9	.5	15	1.5	34	9.1
220	6	1	8	.3	13	.9	25	5.6
340	6	1	7	.2	10	.4	12	1.6
490	6	.1	7	.1	9	.3	12	1.4
680	5	1	6	1	8	.3	10	6
920	5	1	6	1	7	.2	7	.4
Check	9	1.3	15	2.5	30	10.3	45	16.1
SODIUM ARSENITE								
1	8	0.5	21	5.0	31	11.5	39	9.9
2	8	8	18	3.6	25	8.5	35	8.1
4	7	.4	10	1.2	20	2.4	26	3.1
7	7	2	10	8	18	.5	12	8
11	6	2	7	.4	9	.5	8	4
16	6	.1	6	4	6	4	6	.3
22	6	.1	6	2	6	.3	6	2
30	6	.1	6	1	6	2	6	.2
40	6	.1	6	1	6	1	6	1
Check	9	1.4	18	3.8	27	10.2	38	13.9
BORAX								
15	9	1.0	17	2.5	32	10.5	40	12.2
40	8	5	25	3.0	36	9.8	39	8.6
80	8	8	19	3.2	29	5.8	34	6.6
140	7	.3	18	2.6	23	3.6	31	5.6
220	7	.4	13	2.3	21	2.5	27	1.9
340	7	2	10	.7	9	3	9	.7
490	6	.2	8	.3	8	3	8	4
680	6	1	7	3	7	2	7	.2
920	6	1	6	.2	6	1	6	1
Check	14	1.2	18	3.0	34	9.9	45	13.1

The results with borax in water cultures resemble those in soils. Comparison with the concentrations in the water used for moistening the soils shows that toxicity has been reduced from about two times in the Fresno soil to approximately four times in the Stockton soil. This indicates a difference in availability in general agreement with the difference in degree of retention of these two chemicals shown in leaching tests (*4*, *8*).

Chlorate response in solution cultures is similar to that in soils, the toxicity being a function of nutrient level. The changes in toxicity of chlorate as affected by nutrient level, and the interrelations between them, indicate that water-soluble nutrients are the ones involved.

TOXICITY IN CHLORATE-CONTAINING SOIL CULTURES WITH SINGLE-SALT AMENDMENTS

The usual interpretation of the observed relation between soil fertility and chlorate toxicity (*6*) [6] is that the organic matter in the soil reacts with the chlorate to reduce it to chloride. Since, however, the relation between fertility and toxicity holds in water cultures, phenomena other than chlorate reduction are apparently involved. To separate, if possible, the effects of the common nutrient ions, and to study the influence of each alone on chlorate toxicity, the experiments described under Methods (p. 639) and reported in table 6 were performed. This work involved 15 separate treatments on each of 10 different soils, the 9 listed in table 1 and Egbert loam, a semipeat from north of Rio Vista in the Sacramento River delta, a highly fertile soil requiring 170 cc of water per 500-g sample to moisten it to field capacity. Each treatment was represented by a series containing 10 soil cultures with the standard chlorate concentrations. The various ions used are given in table 6, the concentrations being listed on page 639.

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[6] KIESSELBACH, T. A. REPORT OF MEETING OF REGIONAL WORKERS ON BINDWEED CONTROL, SPONSORED BY DIVISION OF CEREAL CROPS AND DISEASES, BUREAU OF PLANT INDUSTRY, U. S. DEPT. AGR. Ames, Iowa. 1936. [Mimeographed.]

TABLE 6.—*Effects of nutrient salts upon chlorate toxicity in 10 California soils as shown by growth of Kanota oats*

[Plants harvested in November and December 1936 and January 1937]

NaClO₃ in air-dry soil (p. p. m.)	Water		2H		NO₃-K		NO₃-Na		NO₃-Ca		K-NO₃		K-Cl		K-SO₄		PO₄-KH₂		PO₄-NaH₂		PO₄-H₃		NO₃-NH₄		NH₄-NO₃		NH₄-Cl		NH₄-SO₄	
	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight
	YOLO ADOBE CLAY																													
	Cm	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*
5	18	1.6	33	10.3	32	7.7	34	9.3	33	7.8	30	5.9	26	3.4	25	3.0	27	4.0	26	3.0	27	4.1	34	8.7	35	9.0	34	7.8	35	9.1
15	20	2.3	34	9.2	36	7.7	31	8.0	33	7.9	28	6.0	23	2.5	23	2.7	22	2.5	25	2.6	26	3.4	33	8.9	32	7.9	35	7.2	33	6.8
40	14	1.2	32	8.2	32	6.7	29	7.1	35	6.3	28	4.7	10	1.4	8	1.1	12	1.0	16	1.4	12	1.2	35	8.7	30	5.2	28	3.9	28	3.9
80	8	.5	30	4.6	27	3.8	28	4.6	29	5.6	20	2.0	4	.3	5	.3	6	.5	7	.4	5	.3	30	6.1	27	3.2	10	1.0	10	1.3
140	5	.4	28	3.0	24	2.5	18	1.5	20	2.6	10	1.2	3	.2	4	.2	4	.2	4	.3	5	.3	28	4.7	13	1.6	4	.3	5	.4
220	4	.2	12	1.4	12	1.2	10	1.0	13	1.5	9	.5	3	.2	3	.2	3	.1	3	.2	4	.2	10	1.5	9	.7	3	.3	4	.2
340	3	.1	10	.9	7	.5	8	.9	10	1.2	6	.5	2	.2	2	.1	2	.1	2	.1	3	2	9	.7	7	5	2	.2	0	.0
490	0	.0	6	.5	6	.5	6	.7	10	.6	3	.2	2	.1	1	.1	0	.0	2	.1	2	.1	6	.5	4	.2	2	.1	0	.0
680	0	.0	6	.4	4	.4	6	.6	6	.4	3	.1	2	.1	0	.0	0	0	0	0	2	.1	4	.2	2	.1	0	.0	0	0
Check	22	1.6	34	10.4	34	9.2	37	9.7	34	9.0	33	6.9	27	4.4	28	3.6	29	4.0	25	3.3	25	4.7	35	10.0	33	9.1	36	8.7	35	9.7
	STOCKTON ADOBE CLAY																													
5	17	1.6	27	9.5	26	6.1	25	6.0	23	5.2	21	4.5	13	1.8	13	1.6	15	1.6	19	3.2	15	1.7	28	7.4	27	7.3	28	7.4	26	6.5
15	12	1.1	27	9.8	22	5.2	25	5.7	24	5.2	22	4.3	15	1.6	14	1.1	13	1.2	16	1.7	13	1.2	28	7.0	27	6.9	25	4.8	27	5.5
40	5	.3	27	10.1	23	4.8	23	5.4	21	4.8	23	3.3	9	.4	9	.3	5	.2	5	.1	6	.3	26	6.4	24	5.3	17	1.4	17	1.4
80	3	.1	27	5.5	19	2.8	23	3.4	25	3.3	12	1.3	4	.1	4	.1	3	.1	4	.1	4	.1	22	4.3	24	2.9	9	.4	6	.2
140	0	.0	23	3.0	21	1.9	19	2.1	18	2.3	8	.4	3	.1	0	0	0	.0	4	.1	0	.0	17	2.1	11	.9	4	.2	3	.1
220	0	.0	5	1.3	10	.9	13	1.2	9	.7	5	.1	0	.0	0	0	0	.0	0	.0	0	.0	15	1.2	10	.4	3	1	3	.1
340	0	.0	4	.2	6	.4	9	.7	8	.4	0	.0	0	.0	0	0	0	0	0	.0	0	0	11	.5	7	.2	0	.0	0	.0
490	0	.0	2	.1	6	.4	7	.2	4	.3	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	7	2	0	.0	0	.0	0	.0
680	0	.0	0	.0	3	.2	4	.1	3	.2	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	5	.1	0	0	0	.0	0	.0
Check	18	2.2	32	10.5	27	6.6	30	6.6	28	6.5	22	4.5	14	2.1	14	2.0	14	1.5	15	2.2	15	2.2	33	8.7	29	7.8	30	8.2	32	8.1

TABLE 6.—*Effects of nutrient salts upon chlorate toxicity in 10 California soils as shown by growth of Kanota oats*—Continued

[Plants harvested in November and December 1936 and January 1937]

AIKEN CLAY LOAM

$NaClO_3$ in air-dry soil (p. p. m.)	Water		2H		NO_3-K		NO_3-Na		NO_3-Ca		K-NO_3		K-Cl		K-SO_4		PO_4-KH_2		PO_4-NaH_2		PO_4-H_3		NO_3-NH_4		NH_4-NO_3		NH_4-Cl		NH_4-SO_4	
	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight
	Cm	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*
5	17	1.7	22	3.8	21	3.5	25	3.8	22	3.6	20	3.0	16	1.8	17	1.6	18	1.9	18	1.8	18	1.5	26	4.0	24	3.6	19	2.5	20	2.7
15	12	.7	23	3.5	21	3.5	23	3.2	19	3.4	19	2.6	15	1.0	13	9	23	1.3	11	.9	10	7	25	3.9	19	2.8	16	1.1	15	.8
40	5	.2	21	2.7	20	2.3	20	1.9	18	2.5	16	1.4	5	.3	7	2	10	.6	7	3	6	3	22	2.9	15	1.4	10	.4	8	.2
80	0	.0	14	1.3	15	1.1	16	.8	13	1.2	9	.7	0	.0	0	.0	4	2	3	.1	5	1	16	1.5	11	7	7	.1	4	.1
140	0	.0	10	.5	7	.5	11	4	9	7	5	.2	0	.0	0	0	0	0	0	.0	0	0	12	.8	7	.3	0	0	0	0
220	0	.0	7	.3	7	.2	7	.2	7	3	0	0	0	.0	0	0	0	0	0	0	0	0	7	.3	5	1	0	0	0	.0
340	0	.0	6	.1	5	.1	5	.1	5	.2	0	.0	0	.0	0	0	0	.0	0	0	0	.0	6	1	0	.0	0	.0	0	.0
490	0	.0	0	.0	0	.0	0	.0	3	1	0	0	0	.0	0	0	0	0	0	0	0	0	0	0	0	.0	0	.0	0	.0
680	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	0	0	0	0	0	0	0	0	0	0	.0	0	.0	0	.0
Check	18	2.3	23	4.0	21	3.4	26	4.0	21	3.4	22	3.0	17	2.2	15	2.0	17	2.4	18	2.4	17	2.4	26	4.4	21	3.1	20	2.8	21	3.1
FRESNO SANDY LOAM																														
5	25	3.3	29	7.8	34	8.1	32	7.5	34	7.4	24	5.0	22	3.1	23	3.0	23	2.7	22	3.5	23	3.8	31	10.9	30	8.7	32	9.0	29	7.2
15	25	2.9	28	7.3	32	8.2	30	6.9	30	6.7	24	4.4	23	2.5	22	2.2	22	2.4	25	3.2	23	3.1	35	10.7	31	7.5	31	7.0	30	6.3
40	17	1.4	26	5.9	34	6.7	30	6.4	30	5.7	25	3.7	15	1.3	9	6	11	1.0	14	1.2	12	1.1	32	8.4	30	5.5	30	4.8	25	4.4
80	9	.4	26	4.7	31	5.0	29	5.3	30	4.5	24	2.7	8	.3	5	1	5	.2	4	1	5	3	27	5.3	29	4.4	25	3.7	21	2.0
140	5	.1	22	2.6	25	2.2	28	3.3	21	1.4	17	1.2	4	.2	0	0	4	.1	3	.1	4	1	26	3.0	21	1.9	15	1.4	13	.8
220	5	.1	19	1.4	16	1.0	10	.7	10	.9	8	.4	3	.1	0	0	0	.0	0	0	0	.0	14	1.1	14	.8	6	.5	6	.3
340	0	.0	12	.5	9	.2	7	.2	6	.1	5	.2	0	.0	0	0	0	.0	0	.0	0	0	13	5	9	.4	5	.2	0	.0
490	0	0	9	.2	6	.1	5	.1	5	.1	4	.1	0	.0	0	0	0	0	0	.0	0	.0	9	3	7	.2	0	.0	0	.0
680	0	.0	3	.1	0	0	0	.0	0	0	0	.0	0	.0	0	.0	0	.0	0	.0	0	0	4	1	0	0	0	.0	0	.0
Check	21	3.4	31	7.4	33	8.2	33	8.1	32	7.3	27	5.2	22	3.4	23	3.1	19	2.8	22	4.6	21	4.3	34	10.0	31	9.1	30	9.7	28	7.9
ARBUCKLE CLAY LOAM																														
5	22	3.4	25	6.9	29	7.9	28	7.8	30	7.6	24	5.2	22	3.0	20	3.4	23	3.4	21	3.1	20	2.9	32	9.4	30	8.3	27	7.3	28	7.8
15	21	2.3	25	6.3	28	7.4	25	7.5	27	7.5	24	5.0	10	1.0	21	1.9	18	2.5	17	2.1	20	2.9	31	9.2	29	7.4	26	5.3	26	4.5
40	7	.4	25	6.2	27	7.1	23	6.0	25	6.3	21	2.8	10	.8	8	.3	7	.5	5	.3	9	.4	28	7.6	29	5.1	8	1.0	7	.4
80	4	.1	25	5.9	22	2.2	21	2.5	25	3.0	10	.7	6	.2	3	.1	4	.1	3	.1	4	.1	28	6.0	11	.8	5	.2	4	.1
140	3	.1	11	1.9	11	.6	10	.6	14	1.7	4	.3	3	.1	0	.0	0	.0	0	.0	0	.0	15	1.2	8	.3	3	.1	0	.0

220	0	.0	10	.8	7	3	7	.3	9	.5	3	2	0	0	0	.0	0	0	0	.0	0	0	8	.3	4	.2	0	0	0	.6
340	0	.0	5	.1	5	.1	4	.1	5	.2	2	.1	0	.0	0	0	0	0	0	0	0	0	4	1	3	1	0	.0	0	.0
490	0	.0	3	.1	0	0	0	.0	3	.1	0	0	0	0	0	0	0	0	0	0	0	0	3	.1	0	.0	0	0	0	.0
680	0	0	0	.0	0	0	0	.0	0	0	0	0	0	0	0	0	0	0	0	.0	0	0	0	.0	0	0	0	0	0	.0
Check	23	3.6	30	8.7	29	8.4	28	7.8	30	8.1	26	5.6	24	3.5	22	3.3	22	3.2	21	3.6	23	3.3	30	10.0	30	8.1	28	7.8	30	8.4

YOLO FINE SANDY LOAM

5	26	6.7	28	8.5	28	7.7	28	7.4	29	8.8	27	8.0	28	7.1	27	7.3	28	6.8	27	6.9	27	7.2	34	9.8	32	8.0	31	7.5	30	9.5
15	25	5.7	30	8.2	30	7.1	27	6.4	29	7.3	28	7.7	27	6.9	27	6.6	27	5.7	25	6.0	28	6.4	31	10.3	32	8.5	27	8.1	35	7.6
40	23	3.2	29	7.7	28	6.0	27	5.9	24	6.4	24	4.9	23	3.3	25	3.6	24	3.9	25	3.4	24	2.8	29	7.7	32	7.1	28	7.0	31	7.6
80	20	1.4	25	4.3	26	4.2	22	3.5	24	3.9	14	1.3	20	1.8	12	9	19	1.8	18	1.9	22	2.1	23	3.5	29	4.5	23	2.7	24	3.5
140	10	.7	22	2.5	23	2.6	18	2.2	20	2.3	9	.6	13	6	6	2	8	5	8	6	11	1.1	22	2.3	23	2.8	15	1.3	16	1.4
220	6	.3	13	1.0	12	1.4	9	.8	13	.9	9	.6	6	3	5	.1	5	2	4	.1	7	.2	14	1.3	10	.8	3	1	3	.2
340	4	.1	6	.3	8	.7	7	4	7	.3	4	2	0	.0	4	.1	4	2	3	.1	0	.0	9	.6	8	.4	3	1	3	.1
490	0	.0	4	.2	7	.5	4	3	4	.1	0	0	0	.0	0	.0	0	.0	0	.0	0	.0	7	3	4	.2	0	.0	0	.0
680	0	.0	4	.2	3	4	3	.1	0	0	0	.0	0	0	0	.0	0	0	0	0	0	0	3	.1	0	.0	0	.0	0	0
Check	30	7.7	29	8.7	31	7.8	28	7.5	32	8.6	32	8.0	28	6.8	30	6.9	27	6.6	28	7.2	27	7.4	32	11.9	32	9.3	31	8.5	34	10.1

EGBERT LOAM

5	27	8.1	34	10.3	28	7.6	28	7.2	32	8.1	27	7.9	27	7.5	27	7.2	28	7.9	31	9.4	32	9.5	33	8.7	31	9.0	32	8.3	32	8.7
15	28	8.2	32	9.0	28	8.1	30	8.3	33	9.5	28	8.6	29	7.3	29	8.2	27	7.0	32	10.2	31	9.0	34	8.6	28	8.6	31	8.4	33	9.0
40	24	5.7	28	7.9	28	7.2	28	7.0	29	8.1	26	7.2	27	6.0	26	6.1	27	4.4	28	6.3	30	7.3	29	7.8	29	7.7	28	6.7	33	7.8
80	16	1.5	30	7.7	27	5.9	27	6.1	26	6.4	27	6.5	29	5.1	21	2.8	17	1.3	23	2.5	23	2.7	29	6.9	24	5.5	25	4.6	26	4.3
140	7	.4	27	5.4	24	4.0	25	4.3	25	5.2	15	1.4	10	1.0	8	4	8	4	8	.5	10	.7	27	5.8	22	3.0	11	1.0	9	1.0
220	4	.1	10	3.2	18	1.1	18	1.6	19	1.8	7	.6	7	.3	5	2	4	1	6	.3	6	2	25	3.1	7	.5	5	.3	8	.3
340	0	.0	7	.3	8	.4	7	.4	9	.5	5	2	4	.1	0	0	0	0	3	2	3	2	11	.8	6	.3	4	3	4	2
490	0	.0	4	.2	5	.2	4	.1	5	.2	5	.1	0	.0	0	0	0	0	3	.1	2	.1	5	3	4	.2	3	.2	0	0
680	0	.0	4	.1	4	1	3	.1	4	.1	0	0	0	0	0	0	0	0	0	0	0	.0	4	2	3	1	0	.0	0	0
Check	30	8.2	33	10.0	31	9.0	30	8.5	32	7.6	27	8.4	29	7.5	28	7.5	28	7.6	32	10.2	31	9.3	30	8.5	29	6.9	31	8.8	34	9.5

COLUMBIA FINE SANDY LOAM

5	30	9.2	32	10.1	36	13.5	33	12.8	35	12.9	30	9.8	33	10.4	29	8.0	30	9.0	32	9.6	32	10.1	34	13.0	32	10.8	32	10.9	33	10.5
15	31	9.1	32	10.8	37	12.4	35	13.4	34	12.9	32	10.5	32	10.1	31	9.1	29	8.3	30	9.8	31	11.1	33	10.9	32	10.6	33	10.7	30	10.0
40	32	8.1	31	9.8	36	11.4	35	12.2	36	10.9	32	9.9	34	10.0	31	7.4	32	7.0	31	8.3	33	8.9	32	10.5	32	7.3	31	7.5	31	6.9
80	27	5.2	31	8.0	35	10.8	34	10.0	34	10.9	31	8.3	31	6.7	28	5.2	26	4.0	27	4.1	29	6.5	29	6.0	26	4.2	23	3.4	23	2.5
140	15	2.2	30	6.6	31	8.0	30	6.4	31	7.7	25	4.8	23	3.4	10	1.0	8	7	17	2.2	23	1.2	26	3.7	22	2.0	15	1.6	9	1.0
220	9	.7	23	3.2	27	5.9	27	4.7	24	3.5	21	2.4	15	1.5	7	.5	3	2	9	8	8	.6	20	2.3	10	1.2	10	.9	7	.6
340	2	.1	12	1.4	14	1.1	9	6	13	1.0	10	8	8	.5	0	.0	0	0	5	3	5	3	13	1.1	8	.7	5	.3	5	.4
490	0	.0	8	.3	7	.5	8	3	10	.5	4	3	4	.3	0	0	0	0	2	2	3	1	8	.6	5	3	4	.2	4	.2
680	0	.0	5	.2	6	.3	7	3	3	2	3	2	0	0	0	0	0	0	0	0	0	0	6	4	3	2	4	2	3	.1
Check	29	8.8	30	10.7	36	12.2	35	12.6	34	13.5	32	11.7	30	8.7	29	8.3	30	8.9	30	9.8	29	10.3	35	14.1	34	11.3	35	12.5	29	10.5

TABLE 6.—*Effects of nutrient salts upon chlorate toxicity in 10 California soils as shown by growth of Kanota oats*—Continued

[Plants harvested in November and December 1936 and January 1937]

YOLO CLAY LOAM

$NaClO_3$ in air-dry soil (p. p. m.)	Water		2H		NO_3-K		NO_3-Na		NO_3-Ca		K-NO_3		K-Cl		K-SO_4		PO_4-KH_2		PO_4-NaH_2		PO_4-H_3		NO_3-NH_4		NH_4-NO_3		NH_4-Cl		NH_4-SO_4	
	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight
	Cm	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*
5	29	6.6	29	11.7	37	10.5	37	10.6	34	8.2	32	9.2	34	11.4	32	10.3	31	9.6	33	9.9	32	9.7	35	14.0	32	10.7	36	14.8	35	10.8
15	30	6.1	29	10.7	37	10.1	36	9.5	33	8.1	32	10.2	32	9.2	33	10.1	33	8.8	33	9.2	35	9.3	31	12.4	32	10.1	35	13.8	35	10.2
40	27	4.0	32	10.0	34	9.3	35	9.8	33	8.1	31	7.8	33	7.5	30	8.7	32	7.9	32	6.6	31	8.9	31	9.8	30	9.2	35	10.2	33	8.6
80	23	2.9	32	8.4	34	8.0	35	8.5	30	7.2	30	6.4	30	6.7	28	5.1	13	1.3	27	3.7	20	1.4	28	7.7	27	6.7	28	5.8	29	5.0
140	14	1.7	31	7.2	30	6.7	32	6.3	28	5.1	25	4.2	23	3.1	10	1.2	10	.7	9	1.5	8	.4	27	5.5	26	4.7	26	4.6	15	4.3
220	9	.9	28	5.8	29	4.9	29	5.2	27	4.3	23	3.1	8	1.4	5	.4	6	.3	6	.2	3	.2	26	4.9	22	3.5	21	2.6	19	2.1
340	7	.3	22	2.8	20	1.5	23	3.4	23	3.2	9	.7	10	1.1	5	.3	5	.2	0	.0	3	.2	21	2.8	11	1.2	15	1.3	15	1.3
490	7	.2	9	.9	15	1.1	15	1.7	17	1.2	9	.7	9	.8	3	.1	3	.1	0	.0	2	.1	15	1.5	9	1.0	7	.7	7	.7
680	4	.1	9	.6	6	.5	9	.9	7	.7	6	.6	6	.3	3	.1	0	.0	0	.0	0	0	8	.8	6	.3	7	.4	6	13.6
Check	33	9.3	33	11.0	35	10.4	34	10.6	34	10.1	31	11.5	33	11.4	33	11.0	32	10.2	30	9.9	32	9.7	34	17.1	35	11.5	35	15.1	33	.4

SACRAMENTO CLAY

$NaClO_3$ in air-dry soil (p. p. m.)	Water		2H		NO_3-K		NO_3-Na		NO_3-Ca		K-NO_3		K-Cl		K-SO_4		PO_4-KH_2		PO_4-NaH_2		PO_4-H_3		NO_3-NH_4		NH_4-NO_3		NH_4-Cl		NH_4-SO_4	
	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight
	Cm	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*
5	37	11.0	38	11.6	43	11.2	45	11.5	42	10.8	37	10.2	36	9.8	39	9.7	41	11.6	35	10.3	38	10.4	41	11.0	41	10.7	42	10.3	45	10.7
15	35	10.0	40	10.8	41	11.2	44	11.0	39	10.3	33	9.8	36	9.7	38	9.3	40	11.0	34	9.4	36	8.5	44	10.7	38	10.6	42	9.4	41	9.3
40	33	7.5	39	10.1	43	9.9	39	9.7	35	9.8	31	8.9	35	7.3	36	7.8	40	9.4	32	8.7	35	7.4	46	10.0	40	9.8	38	7.3	40	8.2
80	30	4.8	38	8.8	34	6.7	37	7.6	28	7.0	28	6.2	29	4.9	30	4.7	37	6.8	31	6.0	29	5.0	41	8.8	36	8.0	31	4.0	28	4.7
140	21	2.4	32	6.0	31	4.1	31	5.2	27	4.5	23	3.4	25	2.7	25	2.3	30	4.2	26	3.3	22	2.8	40	6.7	26	5.0	22	1.9	21	1.8
220	16	1.3	25	3.5	25	2.9	31	3.5	22	2.8	18	1.9	17	1.1	17	1.1	21	2.2	21	1.7	20	1.6	36	4.8	19	2.5	15	1.0	17	.9
340	10	.9	16	2.0	23	2.0	26	2.1	27	1.7	15	1.1	9	.5	11	.5	20	1.0	10	1.0	8	1.0	25	3.2	10	1.1	8	.4	8	.3
490	6	.4	12	1.0	13	1.0	14	1.1	13	1.0	11	.7	4	.2	5	.2	9	.5	10	.5	8	.5	19	1.6	11	.5	5	.3	3	.1
680	5	.1	8	.5	12	.3	13	.8	8	6	9	.3	0	.0	0	.0	3	.2	6	.3	5	.2	14	.8	6	4	0	.0	0	.0
Check	37	11.7	42	11.0	40	11.0	42	13.2	42	10.9	37	10.3	35	9.6	36	9.5	41	11.8	35	11.5	39	10.0	44	12.5	37	10.0	45	10.8	39	10.6

In addition to the data of table 6, the results for certain salts in Yolo clay loam, Stockton adobe clay, and Fresno sandy loam are plotted in figures 9, 10, and 11.

The nitrate curves showed no significant differences between the various salts, the concentration of nitrate being the important factor. In the graphs, consequently, the nitrate values for potassium, sodium, and calcium salts were averaged and the average values plotted. In all cases the toxicity in the nitrate series containing 30 millimols per liter corresponds closely to that in the 2H series containing the same nitrate concentration. Though the absolute values in the Stockton soil are not the same, probably because some other element is further stimulating growth in the 2H series, the values relative to the checks are very similar.

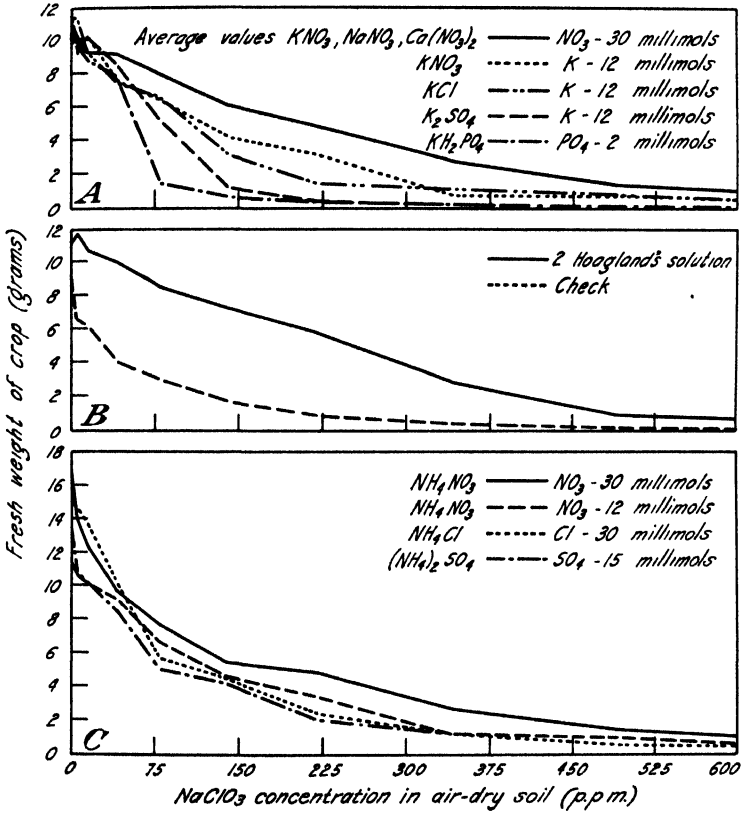

FIGURE 9.—Relation of single-salt applications to chlorate toxicity in Yolo clay loam: *A*, Results of applying various potassium, sodium, and calcium salts individually at concentrations equivalent to those used in Hoagland's solution; *B*, check culture results with chlorate in tap water and in 2H solution; *C*, results with several ammonium salts applied individually.

There is a close relation between nitrate level and chlorate toxicity; the higher the nitrate level, the lower the toxicity. In the potash series in the Yolo clay loam (fig. 9) there is apparently a definite relation between the anions present. Nitrate reduced chlorate toxicity to the greatest extent, followed in order by chloride, sulphate, and phosphate. It has been commonly observed that water-culture plants absorb these anions in this same decreasing order. Evidently chlorate toxicity is related to anion absorption by the plant.

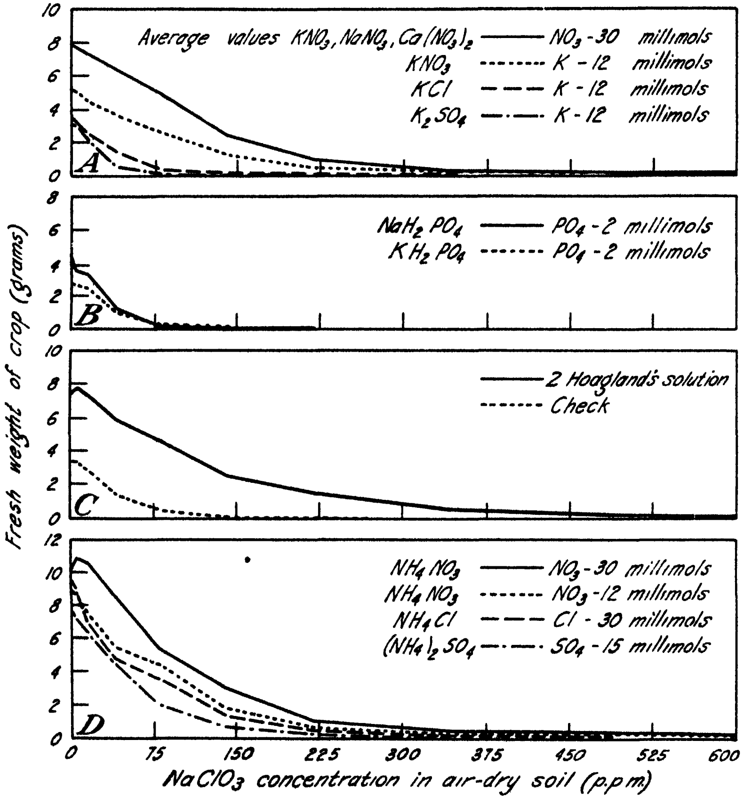

FIGURE 10.—Relation of single-salt applications to chlorate toxicity in Stockton adobe clay: *A*, Results of applying various potassium, sodium, and calcium salts individually; *B*, results of applying the phosphates individually; *C*, controls with chlorate in tap water and in 2H solutions; *D*, results with several ammonium salts used individually.

In the Yolo clay loam all the ammonium salts reduced the chlorate toxicity somewhat as compared with the tap-water check. About a week elapsed between planting and the first inception of chlorate effects in the intermediate toxicity range in these cultures. The plants evidently required some time to absorb a toxic dose of chlorate, and this absorption did not become evident until the first leaves unfolded, except in the very high concentrations. During these days, in the Yolo soil, nitrification of the ammonia evidently took place (*2*) and toxicity declined. Among the ammonium salts the anion relation may be noted in the intermediate toxicity range but is less pronounced than among the potassium salts.

The Yolo clay loam used in these tests is a heavy soil of high fertility. In the graphs of the Stockton adobe clay it may be noted from the check curves showing series moistened with tap water and with double-strength Hoagland's solution that the Yolo soil initially had nearly as much nitrate as did the Stockton soil after it was moistened with the Hoagland's solution. Moistened only with tap water, the Stockton soil produced a very low yield, and toxicity was extremely high, as has been noted before (*4*).

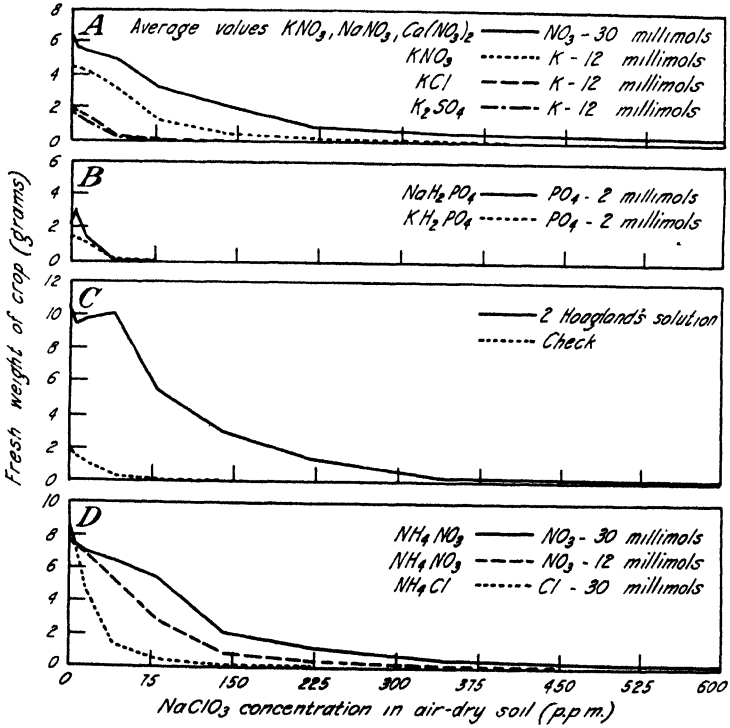

FIGURE 11.—Relation of single-salt applications to chlorate toxicity in Fresno sandy loam: *A*, Results of applying potassium, sodium, and calcium salts individually; *B*, results of applying the phosphates individually; *C*, controls with chlorate in tap water and in 2H solutions; *D*, results with several ammonium salts used individually.

In consequence of the low initial fertility, the yields of the check plants in the Stockton soil (fig. 10) correlate closely with nitrate application, as does chlorate toxicity. It is evident from these curves that potassium concentration has little or no effect upon either crop yield or chlorate toxicity. In this soil of low fertility, furthermore, the nitrate effects far outshadow those of other anions, and the chloride, sulphate, and phosphate curves are not distinctly different.

The curves for the ammonium salts in the Stockton soil differ materially from those of the Yolo clay loam. Though the series show lowering of toxicity related to the two nitrate levels, the chloride and sulphate curves, which, being almost identical, are represented only by the chloride results, show no effect of the ammonium ion upon toxicity. If these curves are compared with the tap-water check and if the results from both are expressed as percentage of the chlorate-free cultures, they will prove to be almost identical. This fact indicates that nitrification in this soil has proceeded slowly, so that the toxic effects of the chlorate had become permanent before any appreciable amount of nitrate was developed. Slow nitrification during the later life of the plants stimulated growth in the lower toxicity range without altering the original toxicity relation. Again, the nitrate effects overshadowed other anions, and no differences due to chloride or sulphate were found.

The Fresno sandy loam is a coarse soil holding less than half as much water at field capacity as the Stockton soil. Since the chlorate is applied in solution with the concentrations based on the air-dry soil, the toxicity should be higher in the Fresno than in the Stockton soil. Examination of the curves (fig. 11), however, shows it to be somewhat lower. Noting the crop yield in the tap-water checks with no chlorate, it is seen that the Fresno soil is somewhat the more fertile of the two; and this fact evidently explains the lower toxicity. As compared with the nitrate level in the soil, water-holding capacity seems to affect toxicity very little.

Though the anion relation in the Fresno soil is in the same direction as in the Yolo, it is less pronounced. This, again, seems to be the case where nitrate effects overshadow those of other ions in soils of low fertility. Among the ammonium salts toxicity has been appreciably reduced by the chloride and sulphate, a fact indicating a more rapid nitrification in this soil, though not so rapid as in Yolo clay loam.

Summarizing the results in the 10 soils from a study of graphs prepared in the analysis of the data, it is evident that nitrate ion has the predominating influence among common soil nutrients in reducing chlorate toxicity. The correlation between the nitrate curves and those for the double-strength Hoagland's solution is evident in all 10 soils. The relation between nitrate level and toxicity reduction is evident from a study of the two levels in the potassium and ammonium salts. Chlorate toxicity is apparently a function of nitrate content in these soils, and any addition of nitrate further reduces toxicity. A single exception exists in the Yolo fine sandy loam, where no distinct difference is shown between the two concentrations of ammonium nitrate added. Perhaps in this highly fertile soil nitrification of the ammonium ions has thrown the results out of line.

Considering next the relation of chloride and sulphate concentration to chlorate toxicity, so evident in the Yolo clay loam, this effect is found to be most pronounced in the fertile soils that show no great response in crop production to fertilization. This tendency for chloride to predominate over sulphate in its effect on chlorate toxicity is very pronounced in the potassium salts in Columbia fine sandy loam, Yolo clay loam, and Egbert loam. It is definitely present in Sacramento clay, Fresno sandy loam, and Aiken clay loam. Among the ammonium salts it is evident in Columbia fine sandy loam, Yolo clay loam, Fresno sandy loam, Arbuckle clay loam, and Aiken clay loam. In the other soils this effect with these salts is either less pronounced or inconsistent. In no case is the relation consistently reversed.

In three of the more fertile soils phosphate ion seems definitely less effective than sulphate in reducing chlorate toxicity; in four soils, including two fertile and two relatively infertile, it seems more effective; and in the three least fertile it shows no consistent relationship. Apparently, therefore, it would be impossible at present to state the effect that phosphate concentration might have on chlorate toxicity, and it would be dangerous to assume, as might be inferred from the results on Yolo clay loam alone, that phosphate increased chlorate effects. One should bear in mind that the phosphate applications in these tests were low as compared with the other nutrients added. To find the effects of these various ions more definitely, it would be desirable to add them in equal concentration instead of in the amounts actually required by the plants. With phosphate this procedure would introduce the problems of solubility and availability. Such studies lie outside the scope of the present work.

Careful analysis of the data on these soils indicates that the cations involved had no primary effect upon chlorate toxicity. The nitrates of potassium, sodium, and calcium were equally effective and showed no consistent cation relationship. Ammonium ion as such had no effect, but when added to soils of high nitrifying power the resulting nitrate was effective. Whether the product of this nitrification affected the toxicity depended on the relative rates of nitrification and chlorate absorption.

The highest nitrification rates occurred in Egbert loam and Yolo fine sandy loam. In these soils ammonium chloride and sulphate materially reduced chlorate toxicity. In Yolo clay loam and Fresno sandy loam smaller changes occurred. In these four soils nitrification of ammonia proceeded so rapidly that nitrate was produced before the plants absorbed enough chlorate to be permanently injured. In the remaining six soils nitrification was not rapid enough to forestall the toxic effects of chlorate; and though crops in five of them were increased, a comparison made on the basis of the checks revealed no change in toxicity. In the Aiken soil there was no evidence of nitrification and the effects of the chloride and sulphate of ammonia differed in no essential feature from those of the potassium salts.

TOXICITY IN CHLORATE-CONTAINING WATER CULTURES WITH SINGLE-SALT AMENDMENTS

Again, to find the nature of soil effects in these studies, water cultures were established as described on page 640. Data on this experiment are presented in table 7 and plotted in figure 12.

The results in culture solution do not differ essentially from those in soils. Since the plants were not large in the single-salt cultures, and since chlorate toxicity was greatly reduced by the higher nitrate concentrations used, the scale has been altered somewhat in figure 12. In calcium nitrate solution containing 60 millimols per liter (equivalent to approximately 4,000 p. p. m. of NO_3), plants survived at a chlorate concentration of 2,040 p. p. m. In the ammonium nitrate culture of this concentration the plants were killed. Even in the lower concentrations some toxicity was evident.

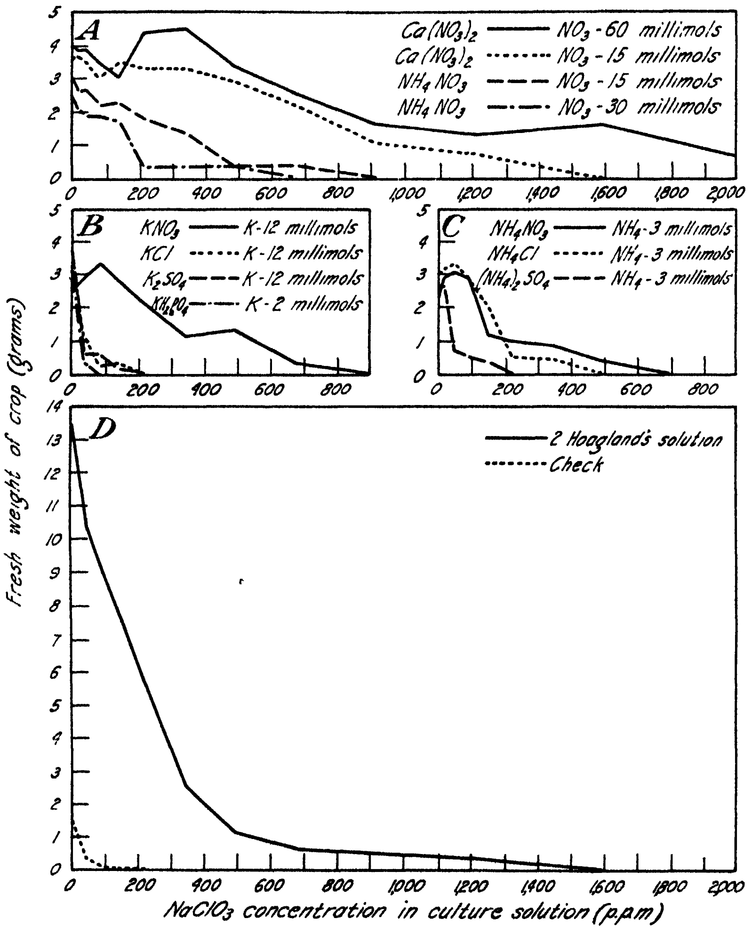

FIGURE 12.—Chlorate toxicity in various single-salt water cultures. The ion concentrations compare with those in the double-strength Hoagland's solution. *A*, Solutions containing calcium and ammonium nitrates; *B*, solutions containing potassium salts; *C*, solutions containing ammonium salts; *D*, controls with chlorate in tap water and in 2 H solution.

TABLE 7.—*Relation of nutrient salts to chlorate toxicity in water cultures as shown by growth of Kanota oats*

[Plants harvested Apr. 30, 1937. Nutrient-salt concentrations expressed in millimols per liter]

NaClO$_3$ in culture solution (p.p.m.)	Checks				Ca(NO$_3$)$_2$				NH$_4$NO$_3$				KNO$_3$		KCl		K$_2$SO$_4$		KH$_2$PO$_4$		NH$_4$NO$_3$		NH$_4$Cl		(NH$_4$)$_2$SO$_4$	
	Water		2H		7.5		30		15		30		12		12		6		2		3		3		1.5	
	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight
	Cm	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*	*Cm*	*G*
15	12	1.0	35	12.5	21	3.7	22	3.8	13	2.5	13	2.0	13	2.6	16	2.6	15	2.7	13	2.1	16	2.9	18	3.2	17	2.7
40	9	3	34	10.3	21	3.5	20	3.8	13	2.6	13	1.8	13	3.0	15	1.2	13	1.7	12	4	16	3.1	18	3.4	13	.8
80	8	.2	34	9.1	18	3.0	22	3.5	12	2.2	13	1.8	13	3.4	12	.3	12	7	0	0	13	2.9	13	2.9	12	.6
140	5	1	33	7.7	19	3.5	19	3.0	12	2.3	13	1.7	13	2.8	12	.4	9	2	0	.0	12	1.2	13	2.1	11	.5
220	0	.0	27	5.6	20	3.3	27	4.4	12	1.8	12	.4	13	2.1	0	0	0	.0	0	0	12	1.0	12	.6	0	.0
340	0	.0	20	2.0	19	3.3	25	4.5	11	1.4	12	.4	12	1.2	0	0	0	.0	0	.0	11	9	11	.5	0	.0
490	0	0	16	1.1	15	2.9	20	3.4	11	.4	12	4	13	1.4	0	.0	0	.0	0	.0	9	.4	0	.0	0	.0
680	0	.0	11	.6	15	2.1	17	2.5	11	4	0	.0	10	.4	0	0	0	.0	0	.0	0	.0	0	.0	0	.0
920	0	.0	10	.5	12	1.0	14	1.6	0	.0	0	0	0	.0	0	.0	0	.0	0	0	0	.0	0	.0	0	.0
1,220	0	0	8	.4	10	.7	13	1.3	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0
1,590	0	0	0	.0	0	.0	12	1.6	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0	0	.0
2,040	0	.0	0	.0	0	.0	8	.5	0	.0	0	.0	0	.0	0	.0	0	0	0	0	0	.0	0	.0	0	.0
Check	12	1.5	36	13.5	21	3.6	28	4.0	13	3.0	14	2.5	13	2.5	16	2.5	17	3.9	18	3.2	15	2.3	17	3.0	19	2.7

Though the toxicity relation between the nitrate, chloride, and sulphate salts seems definite in the ammonium cultures, it is less distinct in the potassium solutions. The phosphate solution showed the highest toxicity of all the single-salt cultures. In viewing these results, however, one should remember that the plants in none of these single-salt cultures were vigorous; and deficiency symptoms in many cases overshadowed chlorate effects. The one outstanding contribution of this final study is the substantiation of the predominating nitrate effect. No matter how fascinating the anion interrelations may be, the practical aspect of all this work is that chlorate toxicity in soils is largely determined by nitrate concentration; and where chlorates are used for weed control the inherent nitrate-supplying power of the soil is of primary importance.[7]

DISCUSSION

With the relation between chlorate toxicity and nitrate concentration so definitely established, the natural inquiry is, How is this effect produced in the plant? Work on this problem is in progress, and eventually the mechanism should be worked out. Meanwhile the results of two preliminary experiments will suffice to answer a major question. Plants of Hubbard squash (*Cucurbita maxima* Duchesne) growing in aerated culture solutions were used to study chlorate absorption under conditions of high and low nitrate in the culture solutions. Plants were grown in each of two tanks, one containing a full Hoagland's solution, the other a one-tenth Hoagland's solution. After about 4 weeks the plants were cut off just below the cotyledons, rubber tubes were slipped over the ends, and xylem exudate was collected after the method of Hoagland.[8] Chlorate was then added to each tank to give concentrations of 100 p. p. m. in each tank, and the chlorate concentrations in successive samples of xylem exudate were determined for about 48 hours. It was found that in the high-nitrate culture little chlorate was absorbed from the solution, whereas in the low-nitrate culture chlorate ions were not only absorbed but concentrated in the xylem sap to many times the concentration of the external culture solution. The effect of nitrate in the culture medium is evidently to inhibit chlorate absorption by roots.

A careful study of the data relating to the general anion relationship indicates that chloride and sulphate ions affected chlorate absorption in all soils, but that the differences were large only in the more fertile soils, where high nitrate content shifted the critical toxicity range into the higher chlorate concentrations. Apparently a relation exists among the anions entering the plant, the probability of any one ion's absorption being determined by its tendency to enter the root as compared with the tendency of all other ions to enter. Since the tendency of one ion to enter would depend upon its concentration and its penetrability, apparently both the physiological activity of the root and the physical and chemical characteristics of the ion are involved. If this assumption is correct, the ability of any one ion to inhibit absorption of another would be related to both these functions, and conditions would determine which predominated. Considering the nitrate, chloride, and sulphate of potassium in the soil cultures, we find the added anion concentration in each case the same; the effect therefore results from the greater absorption of nitrate by the plant. As for the two nitrate levels of the potassium and ammonium salts, the greater tendency to enter, in this case, depends primarily upon the greater concentration of nitrate at the high levels. Consequently, among the anions present in the soil, nitrate would seem to have the predominating effect because it is readily absorbed; and among soils, those having high nitrates show lower chlorate toxicities because nitrate tends to enter the plan'ts from the more concentrated soil solutions to the exclusion of chlorate.

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[7] Just as this paper was ready to submit for publication, the writer's attention was called to a short article by Haines (*10*), who found that both inoculation and ammonium sulphate application improved the percentage survival of red clover seedlings in chlorate-treated soils. Both these treatments evidently increased the nitrate available to the seedlings.

[8] HOAGLAND, D. R., and BROYER, T. C. CERTAIN INTERRELATIONSHIPS BETWEEN ABSORPTION OF SALTS, ROOT PRESSURE, AND TRANSLOCATION OF SALTS. Paper read before the meeting of the American Association for the Advancement of Science, Seattle, Wash. 1936.

That salts other than nitrates may also be involved seems evident from some results obtained on a Wyoming soil particularly high in chlorides and sulphates. Chlorates were reported to have had little effect on plants growing in this soil. A toxicity experiment in the greenhouse proved that this soil had an extremely low chlorate toxicity even though its fertility was not high. Saline soils therefore appear to respond to chlorate applications about as do highly fertile ones, apparently because the plants are unable to absorb chlorates from them.[9]

As these experiments emphasize, absorption of ions is purely a mechanical phenomenon, and utilitarian aspects of this function are secondary. It has long been known that plants will absorb and accumulate large amounts of bromide ion for which they have no use. It now appears that in the absence of nitrate they will absorb chlorate against a concentration gradient and accumulate it to their own detriment.

These results, considered in relation to field experience, clear up several controversial points. (1) Nitrate effects seem to outweigh concentration effects resulting from differences in moisture-holding capacity. For this reason heavy adobe soils, such as the Stockton soil used in these experiments, may show a high toxicity from chlorate, whereas a light soil, such as the Columbia fine sandy loam, may be low.

(2) Organic matter may relate to chlorate toxicity more directly through nitrate effects than through decomposition as has been postulated. Since chlorate has been known to persist in California soils for 3 years or more in semiarid areas and to disappear in wet areas within a year, leaching has apparently more to do with the disappearance of chlorate than has decomposition (*13*). The behavior just reported occurs irrespective of the organic-matter content of the soils in question.

(3) It has been shown here (*3*) that chlorate is more effective during the fall and spring than in summer. A previous publication (*1, p. 34)*, in fact, asserted that chlorate applications should—

> aim to provide a lethal concentration of chlorate in the soil solution during the spring for absorption by the roots during the period when they are depleted of organic foods and engaged in rapid absorption from the soil.

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[9] A previous paper reported the antagonistic action of borate upon chlorate toxicity (7). Probably all anions in the culture medium have some effect, the comparative importance of any one being related to its tendency to enter the plant.

Probably the nitrate content of the soil as affected by leaching during the winter is as vitally concerned as are organic reserves and absorption rate (*5*). Since a definite time is required for absorption and killing by chlorate, not only the content at one time but also the nitrate-supplying power of the soil is involved. Considering the seasonal cycle of nitrification and nitrate absorption by plants, spring seems in all ways the best time for killing with chlorate.

Even more immediate application of the nitrate-chlorate relation may be made. Though a few soils have definite mineral deficiencies, the fertility of most agricultural soils, as shown by vegetative growth of plants, is a reflection of nitrate content. Consequently, the response to chlorate applications may be predicted on most soils by a casual survey of the growing crops and roadside weeds. Although much experience will be required, and such matters as rainfall, species tolerance, and known mineral deficiencies must be considered, this method, when used for prescribing dosages, will eliminate considerable guesswork from chlorate application. In highly fertile soils, furthermore, costly experimentation with chlorate may be avoided and more reliable results obtained with a minimum waste of time by turning immediately to carbon bisulphide or to a cultural or cropping program.

SUMMARY

Although arsenic and boron toxicity are related primarily to textural grade of soils, chlorate toxicity is correlated with fertility.

In a number of soils, increasing the mineral nutrient level invariably decreased chlorate toxicity.

Water cultures of different nutrient levels showed a similar response.

Nutrient level did not affect arsenic or boron toxicity in the high application range either in soils or in water cultures.

In single-salt soil-culture series containing different concentrations of chlorate, those with added nitrate invariably showed lowered chlorate toxicity.

In certain cultures in the more fertile soils, chlorides and sulphates also reduced the toxicity somewhat.

Cations in the culture medium apparently did not affect chlorate toxicity. There was evidence that ammonium ions were nitrified so rapidly in some soils as to affect chlorate toxicity. In most soils, however, nitrification occurred, but went on too slowly to alter the chlorate toxicity.

Single-salt water-culture series containing chlorate showed essentially the same results as the soils.

Absorption experiments with squash plants in high- and low-nitrate aerated culture solutions indicate that little chlorate is absorbed from high-nitrate cultures, whereas chlorate is absorbed in greater quantity and accumulated in the xylem sap in low-nitrate cultures.

Chlorate absorption seems to be a function of root activity and of the concentration and physical and chemical properties of the anions in the culture medium. Under low-nitrate conditions the plant will take up chlorate to its own detriment against a concentration gradient.

Seasonal changes in the response of plants to chlorate application through the soil may be explained on the basis of nitrate concentrations as affected by leaching, temperature, absorption by plants, and nitrification.

Since in most soils fertility, as denoted by the production of crops and roadside plants, is determined by nitrate concentration, plant growth may be used in predicting response to chlorate applications. In highly fertile soils costly experimentation with chlorates may be avoided and a program using carbon bisulphide or cultural or cropping practices substituted.

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SOIL STUDIES ON THE CAUSES OF THE BROWN ROOT ROT OF TOBACCO[1]

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INTRODUCTION

Brown root rot of tobacco has occurred in nearly every area where tobacco (*Nicotiana tabacum* L.) is extensively grown. In Maryland the loss by this disease has varied. In some years the damage has been great, and in others very little has been found. This variation in the intensity of the disease suggested that a study of the environmental conditions might be helpful. This paper reports a study of some of the chemical and biological factors found in soil infected with brown root rot.

HISTORICAL REVIEW

Many causal agencies have been suggested for tobacco brown root rot (*4, 5, 8, 9, 10, 21*).[2] These agencies can be classified roughly as parasitic organisms, toxic substances in the soil, and nutritional disturbances. The following facts suggest that the disease is associated with an organism: The disease can be transferred to some healthy soils by the addition of the infected roots; sterilization destroys the disease; partial sterilization reduces the infection temporarily; heating a soil, so long as the soil micro-organisms are not destroyed, increases the amount of the disease; and the dilution of the diseased soil with healthy soil decreases the infection. However, there were many experimental facts observed in the study of the disease and related field conditions which could not be directly associated with an organism. Attempts to isolate an organism that would reproduce the disease were unsuccessful. Air-drying the infected soil decreased or destroyed the ability of the soil to reproduce the brown root rot. Continuously cropping soil to tobacco caused the disease to disappear.

Since the brown root rot was much worse when certain crops, as timothy (*Phleum pratense* L.) preceded a tobacco crop, it was suggested that the disease resulted from toxic substances left in the soil by some of the previous crops. Aluminum was thought to be one of those important toxic substances in the soil until it was found that application of lime decreased the amount of soluble aluminum and increased the brown root rot. The disease was found to be associated with high organic-matter content and low nitrate content of soils. The apparent control of the brown root rot by the use of large amounts of fertilizer and the ability to bring it back into the soil by applying large amounts of organic materials were also demonstrated. All of these data indicate that the disease is the result of some unbalanced condi-

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[1] Received for publication July 22, 1938.

[2] Italic numbers in parentheses refer to Literature Cited, p. 683

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tion of the soil. This unnatural disturbance may be purely nutritional although it seems more logical that it may be caused by undesirable soil flora.

EXPERIMENTAL PROCEDURE

A study was made of the soil conditions in some badly infected tobacco soils to determine some of the fertility and biological factors associated with brown root rot. These studies consisted of determining the nitrate nitrogen, water-soluble phosphorus, total carbon, and cellulose content. The observations made in the field at each sampling suggested some unnatural activity in the soil flora. In order to test these field observations, many of the soils and infected roots were plated out in different media. A fungus was isolated that would reproduce the disease in healthy soil, when the proper conditions were present. Cultures were made of the fungus to determine its preference in nitrogen, energy, and reaction. Greenhouse tests were conducted to determine the soil conditions required to cause this organism to produce browning of the tobacco roots.

FIELD PLOTS

The field work was conducted on several of the rotation plots at the Marlboro tobacco station and the experimental plots at Cheltenham and Leonardtown, Md. The soil of these plots was sampled in May, June, and July. Nitrate nitrogen, water-soluble phosphorus, total organic matter, and cellulose determinations were made on these samples. Observations on the percent of infection of the brown root rot were made throughout the growing season of the tobacco. There was considerable variation in the nitrate-nitrogen content, especially when different crops had been turned under and different kinds and amounts of fertilizer had been added. The amount of water-soluble phosphorus varied greatly, the most noticeable variation being the effect of fertilizer. The total carbon, and the cellulose content fluctuated widely. There was as much variation between the duplicate plots as between plots receiving different treatments. The results of this study were more or less negative in that it was impossible to associate the presence or absence of the disease with the amount of nitrate nitrogen, water-soluble phosphorus, total carbon, or cellulose. There was a possibility that the dry seasons of 1929 and 1930, when the soil samples were obtained, prevented the proper development of the disease in the field. It is interesting to note that beginning in 1929 there were 5 years in which it was dry during the late spring and early summer. During this time much of the brown root rot of tobacco disappeared in Maryland.

PLATING OUT OF ROOTS

The observations in the field suggested, as they had previously to many other investigators, that the disease was associated with some organism. Accordingly, many samples of infected roots and soils which produced the disease were plated out in several culture media. A large number of fungi were obtained, but the majority of them were the usual rapid-growing fungi secured on soil plates. By accident some of the Czapek's agar plates were left in the incubator much longer than usual before discarding. On examining these plates an entirely different organism was noted.

Pure cultures of this organism were grown and used to inoculate soils and sands that were growing healthy tobacco. In the case of soil that normally produced the disease in the field, the inoculation with pure cultures of this fungus produced the disease even when the soil had been sterilized. The inoculation of healthy soil (which did not normally produce the disease in the field) or quartz sand with cultures of the fungus seldom produced any lesions of brown rot on the tobacco roots. However, if these soils or sand contained considerable organic matter the inoculation of the cultured fungus caused typical brown root rot lesion on the tobacco. These results indicated that this organism is associated in some manner with the brown root rot of tobacco.

DESCRIPTION OF ISOLATED ORGANISM

The fungus was early recognized as being either a *Sclerotium* or a *Rhizoctonia.* John A. Stevenson, senior mycologist of the Bureau of Plant Industry, identified it as *Sclerotium bataticola,* Taub. From the different cultures it was observed that the form-generic names *Sclerotium* and *Rhizoctonia* could quite correctly be used interchangeably for the designation of the sterile stage of this fungus, depending upon the character of the individual colony of the fungus under observation. However, with a few exceptions the fungus was observed to be of the form genus *Sclerotium* and corresponded morphologically to the original description by Taubenhaus (*20*, p. *164*).

> Sclerotia jet black, very minute; exterior smooth, made up of anastomized black hyphae; interior light to dark brown, made up of free, thick-walled cortical, hyphal cells; sclerotia vary much in shape, spherical, oval, oblong, elliptical, curved or even forked, varying in size from 25×22.4μ to 152×32μ, abundant throughout the entire root of the host.

Many workers in other countries described a similar fungus and named it *Rhizoctonia.* In 1925 Briton-Jones (*1*) compared cultures of this *Rhizoctonia* with a culture of *Sclerotium bataticola* received from Taubenhaus and found that they were the same. Therefore, he suggested that the name *Rhizoctonia bataticola* (Taub.) Butler be applied to this sclerotial fungus. This synonym is widely used in the literature outside the United States. Some of these writers designate it *Rhizoctonia* (*Sclerotium*) *bataticola.* A pycnidial stage of a fungus of this type has been referred to as *Macrophomina phaseoli* Ashby. The fungus isolated in this work from soil and tobacco roots was cultured on many media, and no other form developed. Therefore it seems best to call it *Sclerotium bataticola.*

RESPONSE OF ORGANISM TO DIFFERENT ENVIRONMENTAL CONDITIONS

From the observations reported by many of the previous investigators it was concluded that if the brown root rot of tobacco is produced by a parasite, the organism is different from the usual parasite. In order to determine whether *Sclerotium bataticola* had these abnormal characteristics, pure cultures of the organism were subjected to many different environmental conditions. Some of the fungus cultures were dried in the air and in desiccators both within and without the soil, and the effect upon viability was noted. Cultures were grown on many types of media. The relation between the hydrogen-ion concentration and the growth of the organism was studied. The ability of this fungus to utilize various kinds of carbohydrates and different forms of nitrogen was also investigated.

The effect of drying upon the viability of *Sclerotium bataticola* was determined by using diseased soil, healthy soil, and sand. Each sample of soil and of sand was divided into two portions. One lot was for air-drying and the other for incubation under moist conditions. The healthy soil and sand were sterilized and inoculated with cultures of the fungus. The portion of each soil and the sand saved for incubation were made up to optimum moisture and placed in an incubator in the beginning to prevent drying. The remaining parts of the soils and the sand were spread out in a thin layer and air-dried for 4 days. Then each sample was brought to approximate optimum moisture and incubated at 28° C. for 3 weeks. At the beginning of the experiment all soils were plated out on Czapek's solution agar. The fungus was cultured only from the undried portions of the diseased and the healthy inoculated soils and sand. After 2 and 3 weeks of incubation these soils were plated out again on Czapek's agar. With one exception no *S. bataticola* was obtained from any of the soils or sand which had been dried. This exception was thought to be the result of insufficient drying.

Further tests on the effect of drying on the fungus were made. Bits of mycelia were removed from 3-day-old cultures grown in test tubes of sand which had been saturated with Czapek's solution. Each piece of mycelium was air-dried at room temperature for various periods of time and then placed on the surface of Czapek's solution agar in Petri dishes. Ten minutes was sufficient to destroy the viability of the young mycelia. Often 3 minutes of drying prevented growth. The sclerotia which started forming in the test tubes 4 days after inoculation were removed on the 10th day and dried for various periods under different conditions. Some were dried at room conditions, and some in a desiccator over concentrated sulphuric acid. After drying, the sclerotia were placed on Czapek's solution agar and incubated at 28° C. The drying at room temperature rapidly destroyed the viability of the sclerotia; only 1 out of 13 germinated after exposure for 1 hour, and only 1 out of 14 after exposure for 1½ hours. The drying in the desiccator killed the sclerotia much more rapidly. Some of these drying experiments were repeated, mycelia and sclerotia approximately 30 days old being used. The older sclerotia were not completely destroyed until dried for 70 hours or more in a desiccator. This behavior of the organism agreed closely with the results obtained when brown root rot-producing soil was aid-dried.

INOCULATION TESTS

Since previous investigators reported varying degress of infection following different crops it was thought that perhaps the residues in the soil might stimulate this fungus differently. Accordingly, the organism was cultured on several media (*6*), including Czapek's, Waksman's sweetpotato, malt extract, cellulose peptone, ammonia sulphate, and cellulose agars. Except on the Waksman's agar the fungus grew well on all of these media. The colony diameters on the plates were slightly larger on the cellulose peptone agar than on the ammonium sulphate-cellulose agar. This suggested that perhaps the form of nitrogen required by this organism might be very specific.

In order to check this, media containing different sources of nitrogen were made up as suggested by Norman (*13*). Peptone, sodium nitrate, ammonium sulphate, and sodium nitrite were used as sources of nitrogen. The reactions of all the media were adjusted to pH 5.5. Ten plates were poured of each medium, and young sclerotia from Czapek's solution agar were added to each. The results, as presented in figure 1, indicated that *Sclerotium bataticola* seemed to prefer peptone nitrogen although the nitrate nitrogen produced almost as much growth.

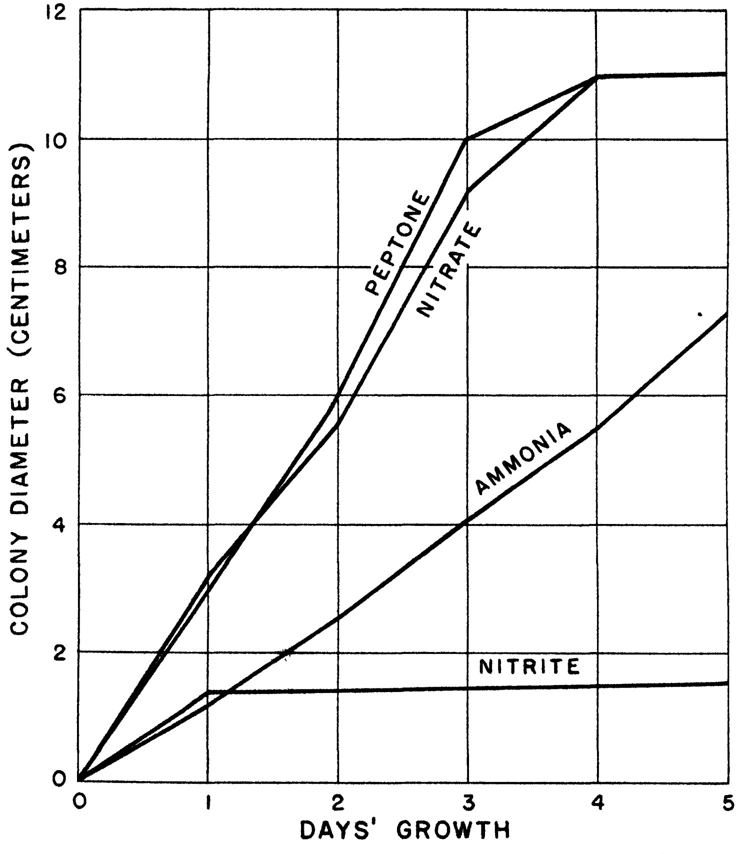

FIGURE 1.—Growth of *Sclerotium bataticola* Taub., as measured by colony diameters, on agar media in which the nitrogen was derived from different sources.

In the tests on the different media, growth was considerably inhibited on Waksman's agar. Since this medium was the only one which had been adjusted to a pH value as low as 4, it was thought that reaction might have considerable effect on the growth of *Sclerotium bataticola*. In order to test this, various portions of Waksman's medium were

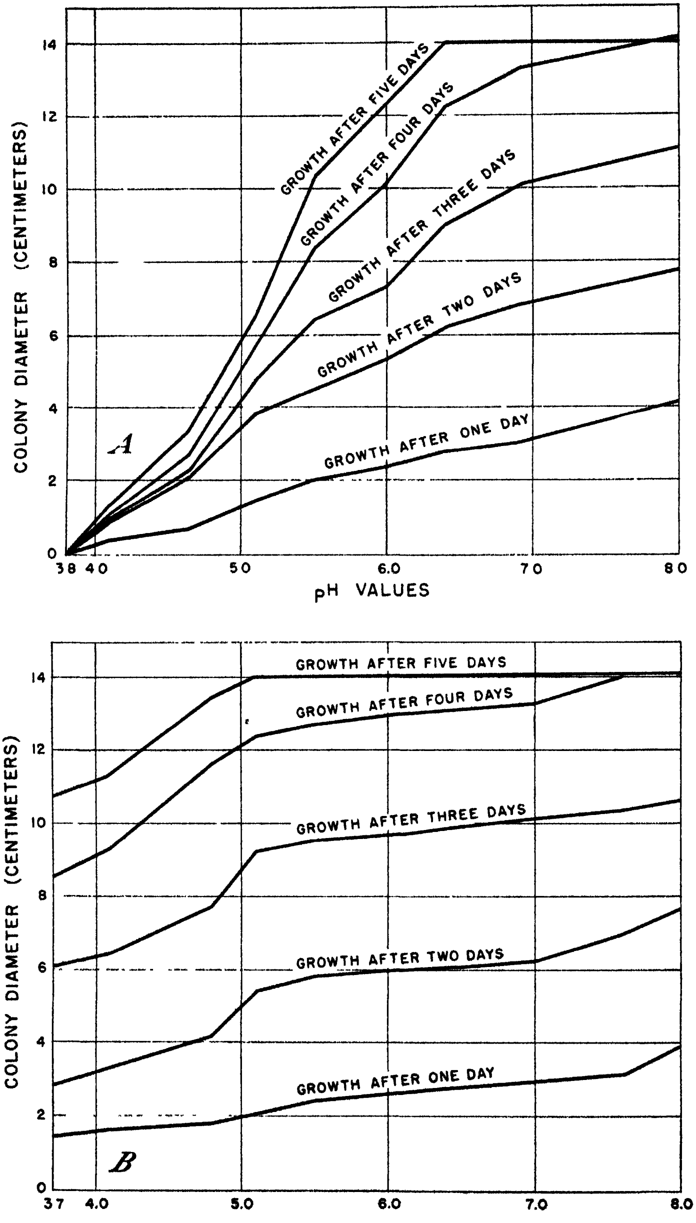

FIGURE 2.—Growth of *Sclerotium bataticola* Taub., as measured by colony diameters, on Waksman's (*A*) and on Czapek's (*B*) agar at different pH values.

made up with pH values varying from 3.8 to 7.9. After the plates were inoculated, they were incubated at 28° C and the colony diameters measured every 24 hours for 5 days. As can be seen in figure 2, *A*, there was considerable difference in the growth. The low pH values retarded the growth very noticeably. The higher the pH value the greater the growth of the organism. Although the optimum reaction was not determined, the results indicated that it would be on the alkaline side of neutrality. This experiment was repeated by using the poorly buffered Czapek's solution agar (*11*). The results as given in figure 2, *B*, are very much different. In the Czapek medium growth was retarded only slightly at the low pH value, although the colony diameter increased with an increase in pH value. It is believed that with this medium the reaction changed quite rapidly under the influence of the growing organisms and the early retardation was quickly overcome.

In some of the previous experiments it was noted that the source of carbohydrates seemed to affect the growth of the organism. Hence the source of carbohydrates was varied; peptone was used as the source of nitrogen. The carbohydrates used were sucrose, lactose, mannite, glucose, potato starch, soluble starch, and cellulose. Each was applied at the rate of 15 g per liter except the cellulose, which was added as precipitated cellulose at the rate of 5 g per liter. The reaction was adjusted to pH 5.6. The results of this experiment showed no significant differences in colony diameter, appearance, growth, or sclerotial forms for the different sources of carbohydrate. This same experiment was repeated by using sodium nitrate instead of peptone as the source of nitrogen. The results were very similar to those of the preceding test, although the peptone media might have had slightly more dense colonies and produced a greater number of sclerotia. From these results it was concluded that *Sclerotium bataticola* is able to utilize several different kinds of carbohydrate equally well.

The apparent ease with which the organism decomposed cellulose led to further test of this ability. Sterilized strips of filter paper in Omeliansky's solution agar (*6*) were readily destroyed by the fungus. Finely ground crude paper was mixed with white sand, sterilized, then moistened with sterilized Czapek's solution, and inoculated with the organism. The paper was rapidly decomposed. However, when an attempt was made to culture the organism on lignin prepared from crude paper, no growth was obtained.

It was apparent from the behavior of *Sclerotium bataticola* in the pure-culture experiments that it could grow well under the conditions observed in the field where the disease were reported. In order to test this hypothesis, several pot tests with tobacco and tomatoes were made. In the first, disease-free or healthy soils were used. The soil treatments, are given in table 1. The organic materials were applied at the rate of 3 tons per acre. The soil was sterilized just before the addition of the inoculant. Young tobacco plants, grown in sterilized quartz sand and nutrient solution, were transplanted to these pots. After a few weeks' growth the plants were removed and the roots examined for the presence of brown root rot lesions. No lesions appeared except where both the filter paper and the inoculant were added. The pots receiving the ground timothy roots made a much better growth. Evidently the sterilizing of this material made considerably more plant food available. This experiment was repeated by using finely ground paper toweling at the rate of 3 tons per acre, with tomatoes as the index crop. Although the roots of the tomato plants after 5 weeks' growth were discolored, they did not have the typical brown root rot lesion.

TABLE 1.—*The influence of timothy residues, cellulose, and the presence of Sclerotium bataticola on the growth of tobacco in pots, and the occurrence of brown root rot in healthy soil*

Treatment [1]	Symptoms of brown root rot	Average dry weight of plants	Relative dry weights
		Grams	
Control	Absent	6.48	100
Inoculated	do	6.60	102
Timothy roots, uninoculated	do	10.52	162
Timothy roots; inoculated	do	3.65	56
Filter paper; uninoculated	do	.45	7
Filter paper; inoculated	Present	.40	6

[1] Organic materials applied at rate of 3 tons per acre. Soils sterilized just before inoculation.

Since brown root rot apparently occurs in the field only on certain soils and under certain conditions, it was thought that perhaps none of these experiments duplicated the exact conditions for the disease. Accordingly, another inoculation experiment was made. This time soil which was known to produce the disease in the field was used. The soil treatments were control, steam steilized, cellulose added, and inoculated, in various combinations, as reported in the following tabulation.

Soil treatment:	*Severity of brown root rot*
Control, no treatment	Moderate.
Steam sterilized	None.
Cellulose added	Moderate.
Cellulose added, sterilized	None.
Inoculated	Moderate.
Sterilized, inoculated	Do.
Cellulose added, inoculated	Moderate to severe.
Cellulose added, sterilized, inoculated	Do.

Cellulose, as finely ground crude paper, was added at the rate of 3 tons per acre. The soils were sterilized for 2 hours after the cellulose had been added and before they were inoculated with cultures of *Sclerotium bataticola*. Tobacco seedlings were grown in these soils for 3 weeks. Brown rot appeared on all the tobacco roots except those in the soils which were sterilized and not inoculated.

The observations of the culture habits of *Sclerotium bataticola* indicated that this organism grew under conditions similar to those reported for brown root rot in the field, also that it could be controlled in very much the same way as in the field. This agreement between the cultural habits and field observations, together with the production of typical root rot lesions in the greenhouse, shows that the disease known as brown root rot of tobacco is produced by the fungus *S. bataticola*.

DISCUSSION

RESPONSE OF ORGANISM TO ENVIRONMENTAL CONDITIONS SIMILAR TO THOSE IN THE FIELD

It is believed that the results of these studies have pointed out fallacies in the previous explanations of causes of this disease. The killing of the fungus *Sclerotium bataticola* by drying, both in the soil and in cultures, agrees with the previous experiments reported upon air-drying of the soil. The authors' inability to plate out the fungus from desiccated soil was also similar to that reported by early workers. The apparent need of this fungus for energy material offers an explanation of the decrease in infection when tobacco is grown continuously. The stimulating effect of lime on the disease in the field correlated well with the tests made upon the effect of hydrogen-ion concentration on the colony diameter of cultures of this fungus. Apparently this fungus is parasitic only under certain environmental conditions. This is in agreement with the results reported by Ciferri (*3*) who showed that any conditions favorable to the luxuriant growth of a *Rhizoctonia* in the soil also favored the parasitism of this organism on the roots of the plants. These observations show that *S. bataticola* could be more parasitic under certain conditions.

Since available organic matter and nitrogen stimulate the activity of this organism, it is believed that this explains the low amount of nitrates in the soil at the time of the greatest activity of the fungus. This belief is further supported by the fact that the soils infected with brown root rot contain considerable plant residues such as cellulose, which has been shown to stimulate the growth of the organism. In the decomposition of these organic materials the fungus probably consumes all of the readily available nitrogen in the soil, and in its search for nitrogen it invades the tobacco roots. The roots of tobacco were found to be very high in nitrogen, especially the nitrate form. This hypothesis, previously suggested by Thomas (*21*), is substantiated by the results obtained when the addition of excessive amounts of cellulosic material nearly always increased the amount of root lesions on tobacco grown in inoculated soils.

EXPLANATION OF FACULTATIVE PARASITISM

The literature indicates further that conditions known to be favorable to brown root rot are similar to, if not identical with, the conditions which produce facultative parasitism of the common endophytic fungi, especially the "*Rhizoctonia*" group. Peyronel (*15*) found that an excess of organic material in the surface layers of the soil favored a luxuriant development of "*Rhizoctonia.*" Extreme moisture conditions were also favorable. This was substantiated by Ciferri (*3*) who showed that any factor which limited the rapid formation of any new roots, or destroyed the equilibrium between root formation and root loss, caused endophytic fungi to become actively parasitic. This was especially true of "*Rhizoctonia*" and similar fungi.

Many of the early workers reported that there seemed to be an association between the root lesions and mycorrhizae in the soil. Ciferri (*3*) indicated this quite strongly in his work with sugar cane. He reported that a "*Rhizoctonia*" was the immediate cause of the root disease and existed endophytically in apparently healthy roots. They became actively parasitic only under certain conditions unfavorable to the plant. He cited Peyronel (*15*) to substantiate these observations. Hopkins (*7*) and Park (*14*) reported that *Sclerotium bataticola* could cause root disease of a number of plants although the fungus commonly existed in mycorrhizal relationship with plant roots. Such a relationship did not seem obligatory. Steinmann (*19*) concluded from his observations that "*Rhizoctonia*" *bataticola* was a common mycorrhizal fungus which under certain conditions became parasitic. He definitely proved this to be the case in the root disease of tea.

A mycelial development within the diseased portion of the tobacco roots was reported by Johnson, Slagg, and Merwin (*10*) early in the study of this disease. The presence of *Rhizoctonia* was also noted by these workers. The stained sections of the roots did not always indicate the presence of an organism. This is in agreement with the work of Steinmann (*19*) who found that hyphae of "*Rhizoctonia*" *bactaticola* were hardly distinguishable in the cells and later apparently disappeared with the advance of the disease on tree roots. Many workers (*2, 12, 14, 18, 19, 22, 23*) have given a large number of host plants for *Sclerotium bataticola*. It is interesting to note that the list of plants susceptible to brown root rot (*10*) contains many plants which other workers have shown to be the host plants. Tobacco has never been listed as a host plant of *S. bataticola* in this country, although it has been in other parts of the world (*2, 16, 17*). Also, this fungus has been associated with the production of other root diseases.

From the results of this study and the observations reported by other workers, it is concluded that *Sclerotium bataticola* can cause brown root rot of tobacco. It has not been proven that this is the only fungus capable of producing the disease. Other organisms of similar morphological and physiological characteristics might be associated with the cause of the disease, although none were isolated in this study. There is a possibility that *S. bataticola* is only indirectly the cause of the disease. Regardless of how this organism produces the root rot infection, it should be remembered that the relation of the chemical, physical, biological, and environmental conditions of the soil to both the tobacco plants and soil flora is very important.

SUMMARY

A study was made of the chemical and biological soil factors which might be associated with the occurrence of brown root rot in tobacco. The nitrate-nitrogen, water-scluble phosphorus, cellulose, and carbon content of the soils were determined on field plots planted to tobacco. Many of the infected tobacco roots and soils were plated out to isolate a fungus which might be associated with the disease. From these cultures an organism (*Sclerotium bataticola*) was obtained which was found to be associated with the disease. The cultural habits of this organism in the laboratory were then studied to see whether its growth habits were similar to those in the field. Finally inoculation experiments were conducted in an attempt to reproduce the disease in the greenhouse. A fungus (*S. bataticola*) was isolated from the infected tobacco roots and from the soil in which these roots grew. From the results of these studies the following conclusions may be drawn: This fungus is readily destroyed by air-drying, it is a cellulose-decomposing organism, it can readily utilize either nitrate or organic forms of nitrogen, and it grows better in neutral to alkaline media.

These characteristics are apparently in accordance with the conditions in the field which influenced the occurrence and severity of the disease. In inoculation experiments in the greenhouse under controlled conditions it was found that *S. bataticola* produced the brown root rot of tobacco.

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A FOLIAR DIAGNOSIS STUDY OF THE INFLUENCE OF CALCIUM FROM TWO SOURCES, LIME AND SUPERPHOSPHATE[1]

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INTRODUCTION

In the preceding paper of this series[2] the course of nutrition of maize plants (*Zea mays* L.) growing on eight plots of tier 1 of the Jordan fertility plots of this experiment station was described.

The treatments of these particular plots consisted of a single element, combinations of two elements, and a complete fertilizer containing all three elements, nitrogen, phosphorus, and potassium. The carrier of nitrogen was dried blood; of phosphorus, the superphosphate of commerce; and of potassium, the chloride or muriate.

Plot No. 1 (check) has never received any fertilizer or lime. But another plot (No. 23), not considered in the previous paper,[2] has received calcium in the form of lime, without any commercial fertilizer, every 4 years at the rate of 500 pounds per application to the ⅛-acre plot. Four of the plots, P (No. 3), NP (No. 5), PK (No. 7), and NPK (No. 9), received calcium in the form of ground bone black until 1917. Since then they have received superphosphate at such a rate as to provide 48 pounds of P_2O_5 to the acre, applied to the corn and wheat. Plot No. 23 accordingly received 3,000 pounds of calcium in the form of lime (CaO) up to 1936, and plots Nos. 3, 5, 7, and 9 received during the same 56-year period (1881–1936) approximately 400 pounds of calcium present in the superphosphate and ground bone black.

The object of the present investigation was to compare the foliar diagnosis of corn plants growing on a plot receiving lime only with that of corn plants on a check plot receiving no lime, and to determine the relationship of the foliar diagnosis of plants growing on plots receiving calcium in the form of superphosphate to that of plants on a plot receiving lime only. The results are reported herein.

EXPERIMENTAL PROCEDURE

The arrangement of the experimental plots has been described previously.[2]

The sampling of the leaves was carried out in the manner described in detail in an earlier paper.[3] The third leaf from the base of the stalk was taken periodically during the season from all of the plants lengthwise of each plot. Successive rows were sampled on each of the sampling dates indicated in table 1.

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[1] Received for publication August 29, 1938. Paper No. 857 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

[2] THOMAS, WALTER, and MACK, WARREN B. FOLIAR DIAGNOSIS OF ZEA MAYS SUBJECTED TO DIFFERENTIAL FERTILIZER TREATMENTS. Jour. Agr. Research 58: pp. 477–499, illus. 1939.

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PRESENTATION AND DISCUSSION OF RESULTS

The composition of the third leaf expressed as percentages of N, P_2O_5, K_2O, CaO, and MgO in the dried foliage and the composition of the NPK unit [2] and yields of grain and stover of plants from plots No. 1 (check) and No. 23 (lime), are listed in table 1 and shown graphically in figures 1 to 3.

For purposes of orientation the derivation of the NPK unit represents the equilibrium between N, P_2O_5, and K_2O in the chosen leaf at the moment of sampling and is derived by converting the percentage composition for N, P_2O_5, and K_2O into milligram-equivalent values and then determining the proportion each of these bears to the milligram-equivalent total.[3]

COMPARISON OF THE FOLIAR DIAGNOSIS OF PLANTS ON THE LIME PLOT (NO. 23) WITH THAT OF PLANTS ON THE CHECK PLOT NO. 1

INDICATIONS GIVEN BY THE GRAPHS SHOWING THE PERCENTAGE OF N, P_2O_5, K_2O, CAO, AND MGO AS ORDINATES AND DATES OF SAMPLING AS ABSCISSAE

TABLE 1.—*Percentage composition expressed in terms of the dried foliage together with the composition of the NPK unit and yield of plots No. 1 (check) and No. 23 (lime)*

Date and plot	N	P_2O_5	K_2O	$N+P_2O_5+K_2O$ (s)	CaO	MgO	Composition of the NPK unit (relative proportions of N, P_2O_5, K_2O)			Yield per plot	
							N	P_2O_5	K_2O	Grain	Stover
	Percent	*Percent*	*Percent*	*Precent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Pounds*	*Pounds*
Check No. 1											
July 6	2 960	0.307	2.387	5 654	2 308	0 934	76 804	4 719	18 477	165.4	117 1
July 21	2.210	.305	2.515	5 030	2 087	966	70 361	5 753	23 886		
Aug. 8	1.800	230	1.841	3 871	1.605	1.039	72 421	5 482	22.097		
Aug. 25	2.010	316	1.608	3 936	1 999	1 216	75 087	6 993	17 920		
Lime No. 23											
July 6	3.260	582	872	4 714	4.144	1 376	84 348	8 921	6,731	452 2	222 1
July 21	2 710	.568	910	4.188	4 233	1.231	81 676	10 142	8 182		
Aug. 8	2 410	474	910	3 794	3 851	1 122	81 356	9 480	9 164		
Aug 25	2 340	524	891	3 755	3 983	1 014	80.241	10.645	9.114		

In the following discussion it is necessary to refer continually to the content of, or the graph for, nitrogen, phosphoric acid, potash, lime, or magnesia of the selected leaf (third from the base) of plants growing on a particular plot. To avoid monotonous repetition and a cumbersome structure, the plot treatment or its symbol (check, N, P, lime, etc.), is used to refer to leaves from plants growing on the plot indicated.

NITROGEN

The graphs for nitrogen are unlike both in form and position; the graph of lime (No. 23) is higher than that of the check (No. 1) throughout the whole period. At the third sampling the difference is as great as 0.61 percent.

PHOSPHORIC ACID

The graphs for phosphoric acid are similar in form but not in position. The graph of lime (No. 23) is much above that of the check (No. 1) throughout the whole period, an indication that inhibition of the absorption of P_2O_5 resulting from the medium (soil) has been removed by liming. The mechanism of this phenomenon has been described.[4]

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[2] THOMAS, WALTER. See footnote 3, page 685.

[3] THOMAS, WALTER, AND MACK, WARREN B. See footnote 2, page 685.

POTASH

The graphs of potash are unlike both in form and position. The graph of lime (No. 23) is always much below that of the check (No. 1). During the early period the K_2O content of the selected leaf from plants on plot receiving lime (No. 23) is less than one-half that of the selected leaf from plants on check plot (No. 1). Lime, consequently, has reduced the absorption of potassium by the plant, and the seat of this inhibition lies in the soil and not in the plant.

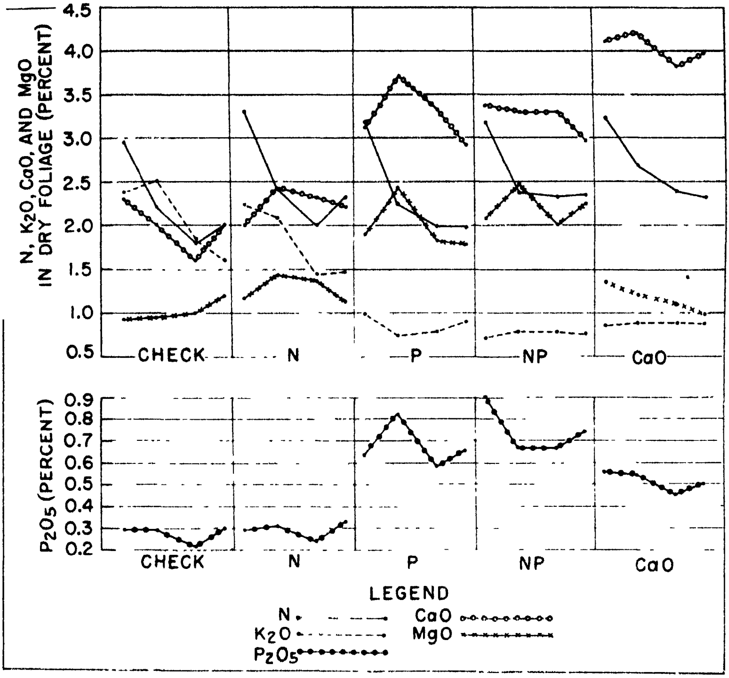

FIGURE 1.—The nitrogen, phosphoric acid, potash, lime, and magnesia content of the third leaf plotted as ordinates, and dates of sampling as abscissae. (The dates of sampling are July 6, July 21, August 8, and August 25, and are indicated by the four dots in each curve.)

LIME

The graphs of lime differ in form and position. The graph of lime (No. 23) is very much higher than that of the check (No. 1) throughout the whole period, a reflection of the fact that certain functions of potassium can be performed by calcium when the former is deficient.[5]

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[4] THOMAS, WALTER. PROPERTIES OF THE HYDROXYL GROUPS OF CLAY AS A BASIS FOR CHARACTERIZING A MINERAL SOIL. Soil Sci. 42: 243–259, illus. 1936.

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MAGNESIUM

The graph of the check (No. 1) rises progressively, whereas that of lime (No. 23) falls progressively with the advancing age of the leaf. Such relative differences between import of Mg into the leaf and export from it are an indication that the demand by the plants on the former plot is always less, and on the latter always greater than the supply.

INDICATIONS GIVEN BY THE GRAPHS SHOWING THE NPK UNITS IN TRILINEAR COORDINATES

The composition of the NPK units at the various sampling dates is given in table 1. The mean intensity of nutrition and the mean NPK units are given in table 2, and the latter are shown graphically in figure 2.

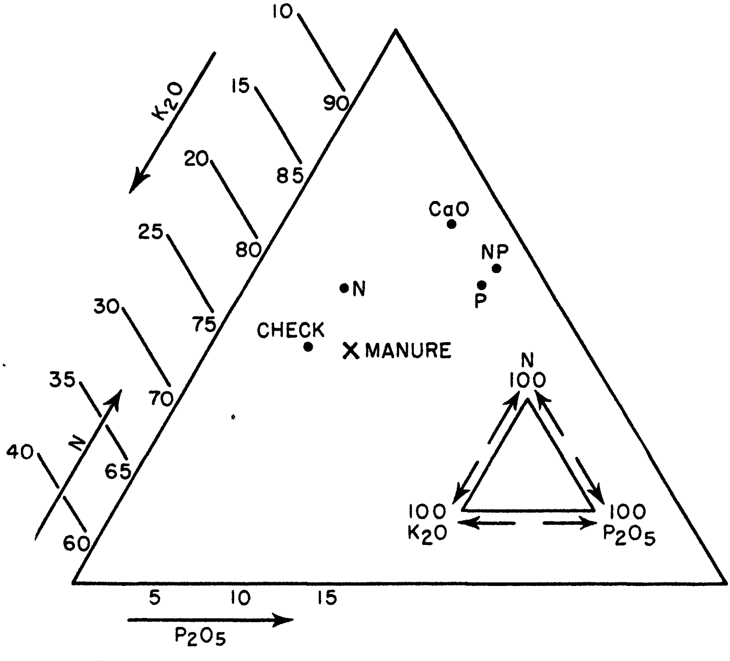

FIGURE 2.—Positions of the mean NPK units of the third leaf from the check plot, and from plots receiving dried blood, superphosphate, and lime, compared with that of the optimum (manure+CaO).

Liming reduced the intensity of nutrition by its effect in reducing the absorption of potassium. The effect of liming on the N—P_2O_5—K_2O equilibrium was to increase the nitrogen of the NPK unit at the the expense, principally, of the potassium.

In figure 2 the position of the mean NPK unit of the treatment resulting in the highest yields of the plots on tier 1, namely manure+CaO, is marked by a cross (×). Check (No. 1) has a lower intensity than has manure+CaO (No. 22), with insufficient P_2O_5 and a slight excess of K_2O in the NPK unit. Lime alone resulted in reducing the intensity below that of the check (No. 1). The notable effect of liming is on the composition of the NPK unit, the K_2O of which is reduced from a value of 20.595 in the check plot (No. 1) to 8.298 in the lime plot (No. 23), and the P_2O_5 increased by more than 70 percent.

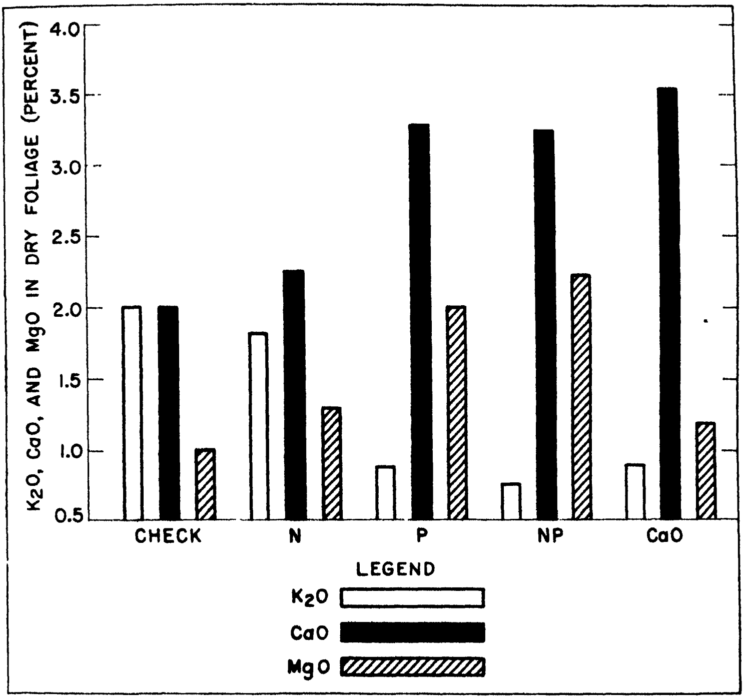

FIGURE 3.—The mean content of potash, lime, and magnesia, respectively, of the third leaf of plants from the check plot, and from the plots receiving dried blood, superphosphate, and lime.

TABLE 2.—*The mean intensity of nutrition, the mean NPK unit and yield of plot No. 1 (check) and plot No. 23 (lime), and plot No. 22 (manure)*

Plot No.	Treatment	Intensity of nutrition	Composition of the mean NPK unit [1]			Yield of grain per plot
			N	P_2O_5	K_2O	
			Percent	*Percent*	*Percent*	*Pounds*
1	Nothing	4.62	73.668	5.737	20.595	165.4
23	Lime	4.11	81.905	9.797	8.298	452.2
22	Manure	5.88	73.376	8.234	18.390	770.8

[1] The values of the mean intensity of nutrition and of the mean NPK unit for the respective treatments are the mean of the values for the intensities and NPK units, respectively, at the four dates of sampling recorded in column 5 and in columns 8, 9, and 10 of table 1.

COMPARISON OF THE FOLIAR DIAGNOSIS OF PLANTS GROWING ON PLOTS P (NO. 3), NP (NO. 5) AND LIME (NO. 23) WITH THAT OF CHECK PLOTS (NO. 1) AND N (NO. 2)

INDICATIONS GIVEN BY THE GRAPHS SHOWING THE PERCENTAGE OF N, P_2O_5, K_2O, CAO, AND MGO AS ORDINATES AND DATES OF SAMPLING AS ABSCISSAE

The K_2O graphs of P (No. 3), NP (No. 5), and lime (No. 23) are characterized by relatively small changes with advancing age of the leaf. The K_2O graph for lime (No. 23) differs but little either in form or position from the graphs for K_2O of P (No. 3) and of NP (No. 5), which are the lowest of any treatments in tier 1.[6] The comparisons are brought out better in table 3 and figure 3, which show the mean percentage composition with respect to K_2O, CaO, and MgO, respectively, at the four sampling dates.

TABLE 3.—*Comparison of the foliar diagnosis and yield of plants on the P plot (No. 3), NP plot (No. 5), and lime plot (No. 23) with check plot (No. 1) and N plot (No. 2)*

Plot No.	Treatment	K_2O	CaO	MgO	Yield of grain per plot
		Percent	*Percent*	*Percent*	*Pounds*
3	P	0.87	3.29	2.00	371.2
5	NP	.75	3.26	2.23	376.7
1	Nothing	2.09	2.00	1.03	165.4
2	N	1.82	2.26	1.29	265.6
23	CaO	.89	3.55	1.18	452.2

The addition of phosphate only and also of phosphate combined with nitrogen (NP) has resulted in a reduction of the potash content of the leaf by one-third of the values found in the check (No. 1) and also in N (No. 2). Liming has produced effects quantitatively similar. The conclusion is that the element common to both treatments calcium—is the causal factor.

LIME AND MAGNESIA

In a preceding paper,[6] it was shown that the CaO graphs of P (No. 3) and also of NP (No. 5) were much higher than that of any of the other treatments without lime, viz. (check, N, K, NK, PK, NPK), and that throughout the whole period, the CaO content of the leaf from plots receiving superphosphate without potash (P, NP), were only slightly lower than the CaO content of the leaf from the limed plot, whereas the MgO content was actually greater. McIntire[7] stated many years ago that—

> the application of phosphorus in the form of dissolved bone black (used as the carrier of phosphorus prior to 1890) carries at least one-half as much $CaSO_4$ as is applied on plat 33 (gypsum only), and it is possible that part of the increase upon the phosphorus treated plats is due to the lime or sulphur in the gypsum contained in the dissolved bone material * * *.

But the element sulphur is not present in the lime added to plot No. 23, which fact eliminates this factor from consideration here.

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[6] THOMAS, WALTER, and MACK, WARREN. B. See footnote 2.

[7] MCINTIRE, W. H. RESULTS OF THIRTY YEARS OF LIMING. Pa. Agr. Expt. Sta. Ann. Rept. 1911-12: 64-75. 1913. See p. 71.

The writers indicated in the previous paper[8] that the CaO and MgO graphs of plots PK (No. 7) and NPK (No. 9) which received both superphosphate and potash were below those of plots P (No. 3) and NP (No. 5) which received superphosphate without potash. Their findings give additional confirmation of the reciprocal effects of potassium and calcium and of the fact that the seat of this influence is located in the soil and not in the plant.

INDICATIONS GIVEN BY THE GRAPHS SHOWING THE NPK UNITS PLOTTED IN TRILINEAR COORDINATES

The mean intensities and the mean NPK units of the third leaf are shown in table 4 and graphically in figure 2. The calcium of superphosphate has had a depressing effect on the intensity of nutrition (table 4, column 3) just as lime has had.

The mean NPK units (fig. 2) of plots P (No. 3), NP (No. 5), and lime (No. 23) are displaced relatively higher up and farther toward the apex of the triangle representing $P_2O_5 = 100$ percent than the others, indicating a low K_2O and a high P_2O_5 in the NPK unit.

The relative positions of the check plot (No. 1) and of the plot receiving manure + CaO (No. 22), the lowest and highest yielding plots, are interesting. The composition of the NPK units with respect to N is nearly identical in both; with respect to K_2O the difference is not very great. The great differences in the yields of these two treatments are associable with relatively large differences in the intensity of nutrition and in the composition of the NPK unit with respect to P_2O_5 which is nearly 50 percent higher in plot receiving manure (No. 22).

TABLE 4.—*Comparison of the mean intensities of nutrition, mean NPK-units, and yield of P (plot No. 3), NP (plot No. 5), and lime (plot No. 23), with nothing (plot No. 1) and N (plot No. 2)*

Class order	Plot No	Treatment	Intensity of nutrition	Composition of the NPK units			Yield
				N	P_2O_5	K_2O	
				Percent	*Percent*	*Percent*	*Pounds*
3	3	P	3.97	77.84	13.64	8.52	371.2
	5	NP	4.13	78.84	13.92	7.24	376.7
4	1	Nothing	4.62	73.67	5.74	20.59	165.4
	2	N	4.67	77.62	5.77	16.61	265.6
2	23	Lime	4.11	81.90	9.79	8.29	452.2
1	22	Manure	5.88	73.38	8.23	18.39	770.8

The importance of P_2O_5 is shown by the P plot (No. 3) as compared with the check plot (No. 1). A reduction of P_2O_5 in the NPK unit from 13.64 to 5.737, or approximately 58 percent, brought about a reduction in yield of grain, regardless of the simultaneous increase of K_2O in the NPK unit from 8.52 to 20.595, or approximately 142 percent, which occurred principally at the expense of the P_2O_5, though to a lesser extent at the expense also of the N in the NPK unit.

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[8] See footnote 2, p. 685.

(C) THE RECIPROCAL EFFECTS OF CALCIUM AND POTASSIUM

The absorption of an element does not depend only upon the quantity of that element in the fertilizer, but on its quantitative relations to the other elements, even if there is not an absolute deficiency of them. Conclusions cannot, therefore, be drawn with respect to the nutrition of a plant from experiments in which the quantity of a single fertilizer element applied to the soil is the variable.

The addition of calcium in the form of superphosphate or in the form of lime lowers the level of nutrition with respect to potassium, producing very low values for K_2O in the NPK units. The causes lie in the changes in the equilibrium between K and Ca in the medium (soil), which result in a lower concentration of potassium in the soil.[9] This condition is reflected by the leaf with great sensitivity. Hence, in passing from a plot in which calcium dominates over potassium to a plot in which potassium dominates over calcium, the leaf faithfully reflects this inversion.

The effect on yields depends upon the level to which K or Ca has been reduced in the leaf. The mean K_2O content of the leaf in the optimum treatment—manure+CaO (No. 23)—is 2.44 percent, and the addition of calcium to the fertilizers on other plots, whether as superphosphate or as lime without potash additions, has reduced the level of K_2O in the leaf considerably below this optimum level. This reduction is a causal factor contributing to the reduced yields.

Thus through a deficiency of the medium (soil) in a certain element, the plant reacts by increasing the absorption of another element—an indication of the great changes possible (plasticity) in the composition of the plant.

SUMMARY

The nutrition of *Zea mays* growing on long-continued fertility plots receiving calcium from two sources, lime and superphosphate, is compared with that of plants on (1) a check plot receiving no fertilizer and no lime, (2) an unlimed plot receiving nitrogen only without superphosphate or potash, and (3) unlimed plots receiving superphosphate and potash with and without nitrogen (NPK, PK).

The results are recorded by means of graphs showing (1) the percentages of N, P_2O_5, K_2O, CaO, and MgO (as ordinates) and dates of sampling (as abscissae), and (2) the equilibrium between N, P_2O_5, and K_2O and the intensities of nutrition at the moment of sampling.

The effects of calcium from the two sources, lime and superphosphate, were similar, resulting in increased absorption of native soil nitrogen and depressed absorption of native soil potassium as well as of added potassium, with concomitant increases in the absorption of calcium and magnesium.

Calcium from both sources reduced the intensity of nutrition with respect to nitrogen, phosphoric acid, and potash ($N+P_2O_5+K_2O$) and also lowered the K_2O in the NPK unit from 20 to 8 (in round numbers).

Liming resulted in an increase in the nitrogen of the NPK unit and in a relatively great increase in the P_2O_5 made at the expense, principally, of the potash.

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[9] THOMAS WALTER. THE DISTRIBUTION AND CONDITION OF THE POTASSIUM IN A DIFFERENTIALLY FERTILIZED HAGERSTOWN CLAY LOAM SOIL PLANTED TO APPLE TREES IN CYLINDERS. Jour. Agr. Research 53: 533-546. 1936.

The yields of grain from the different treatments are classed in relation to the intensities of nutrition and the composition of the mean NPK units.

The lowest yields of grain are associated not with treatments (check, dried blood) having the lowest intensity of nutrition, but with the lowest P_2O_5 in the NPK unit. A reduction of 58 percent in P_2O_5 in the NPK unit of the check as compared with P brought about a reduction in yield of grain not offset by the accompanying increase of 142 percent in K_2O in the NPK unit which occurred at the expense, principally, of P_2O_5.

MONTHLY VARIATION IN CAROTENE CONTENT OF TWO IMPORTANT RANGE GRASSES, SPOROBOLUS FLEXUOSUS AND BOUTELOUA ERIOPODA [1]

By W. E. WATKINS [2]

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INTRODUCTION

Much of the semiarid mesa land lying in the south-central part of New Mexico along the Rio Grande has an average annual precipitation of from 8½ to 10 inches. Approximately half of this precipitation usually occurs in July, August, and September, the primary growing season during which most of the range grass is produced. Two of the most important range grasses of this area are black grama and mesa dropseed grass. These two grasses were selected in 1936 for a study of their nutritive properties at different stages of maturity.

Black grama (*Bouteloua eriopoda* (Torr.) Torr.), one of the very palatable grasses for livestock, grows on the mesas, hills, and dry open ground of large areas of range land in Texas, New Mexico, Arizona, and southern Utah. The upright stems of this grass remain green for a distance of 4 to 6 inches from their bases throughout the winter. Mesa dropseed grass (*Sporobolus flexuosus* (Thurb.) Rydb.) is found on the mesas of western Texas, New Mexico, Arizona, southern Utah, and southern California. It grows to a height of 20 to 30 inches and is very palatable throughout the growing season.

MATERIALS AND METHODS

The two grasses, black grama and mesa dropseed, were collected on the 15th of each month from an enclosure on a ranch of the New Mexico College of Agriculture and Mechanic Arts, located approximately 22 miles north of Las Cruces. The rainfall data were obtained from a rain gage on the experimental plot. The first sample was collected February 15, 1937, and similar collections will be made monthly until the completion of the project, which was planned to cover a period of 5 years. The bunches of grass in the plot were staked out in series of 12. The first series was harvested only on January 15 of each year and represents grass in an ungrazed pasture. The sixth series was harvested June 15 and contained some mature grass that had grown since June of the year before, along with new grass of the current season. The October series contained only the current year's growth at its approximate maturity. The black grama was cut 2½ inches and the mesa dropseed 3 inches above the surface of the ground. The samples were placed in canvas bags, weighed, taken to the laboratory, and subjected to analysis within an hour or an hour and a quarter after cutting. Occasionally, when it was not possible to begin the analysis

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[1] Received for publication September 6, 1938.

[2] Acknowledgment is due R. W. Caldwell, of the California Agricultural Experiment Station at Davis, Calif., for valuable assistance in connection with certain phases of the project and for criticizing the manuscript.

immediately, the samples were placed in a mechanical refrigerator at a temperature of −3.0 to +1.0° C. and analyzed the following morning.

All the samples were finely pulverized in a Wiley mill except those containing fresh green growth with a moisture content of 50 percent or over, which were ground in a mortar with fine sea sand. All the samples, regardless of the method of grinding, were analyzed without drying as soon as they could be transported to the laboratory and prepared.

The plants were analyzed for carotene by the method of Guilbert,[3] with modifications suggested by R. W. Caldwell. Seven months after the beginning of this project, Munsey [4] published the results of his referee work on carotene methods. From reports and comparisons by other workers it is believed that the method used in the present work gives results that very closely approximate those obtained with the Fraps-Guilbert method used in the referee work. A 92-percent methyl alcohol wash solution was used instead of the 90-percent solution specified by the Guilbert method. Briefly, the procedure consists in subjecting the pulverized sample to saponification in aldehyde-free alcoholic potassium hydroxide and extraction with peroxide-free ethyl ether. The ether solution, after washing free from chlorophyllins, flavones, and alkali, is distilled under reduced pressure, the residue taken up in petroleum ether (boiling point, 35°–60° C.), and the xanthophyll removed by washing with 92-percent methanol. The carotene in petroleum ether solution, after making up to convenient volume, is determined colorimetrically, a dye solution prepared from orange G and naphthol yellow crystals being used for comparison. The dye solution was standardized against a sample of β-carotene [5] having a melting point of 184° C. Duplicate determinations were made upon the two range grasses and very close checks were obtained. The error averaged less than 4 percent.

ANALYTICAL RESULTS

The results of the analyses, presented in table 1 and figure 1, show that in 1937 the carotene value for black grama increased above the February and March levels to a high level in June, and then fell somewhat during July and August. This July and August period was rather dry and the grass made little growth, which may account for its low carotene content. The September value, which is the highest of the year, can be attributed to the fresh green growth that followed heavy early September rains. This growth continued at least until the middle of October, when the carotene value was approximately 15 percent less. The carotene values declined during the early winter months to a level of approximately 15 mg. per kilogram during February and March 1938. The black grama grass during February and March 1938 contained approximately three times the carotene content present during the same months of 1937. The winter of 1937–38, so far as minimum temperatures are concerned, was very mild. According to the records of the United States Weather Bureau station at State College, N. Mex., the average minimum monthly temperature for October 1936 and the three following months was

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[3] GUILBERT, H. R. DETERMINATION OF CAROTENE AS A MEANS OF ESTIMATING THE VITAMIN A VALUE OF FORAGE. Indus. and Engin. Chem., Analyt. Ed. 6: 452–454 1934.

[4] MUNSEY, V. E. REPORT ON CAROTENE. Jour. Assoc Off. Agr. Chem. 20: 457–458. 1937.

[5] This sample was supplied through the courtesy of Dr. H. H. Strain, of the Carnegie Institution of Washington.

42.6°, 32.2°, 27.9°, and 21.4° F., respectively; the minimum monthly temperatures from October 1, 1937, to April 1, 1938, averaged 2.13° F. warmer than for the same period of 1936 and 1937. It is believed that this difference in temperature permitted a larger fraction than usual of the upright stems to remain green during the winter and thus furnished plant material with a higher carotene content.

Guilbert and his coworkers [6] found the minimum carotene requirement for cattle, sheep, and hogs to be 25 to 30 micrograms daily per kilogram of body weight. On this basis, the black grama grass contained, at its lowest level during February and March 1937, over three times the minimum amount of carotene needed for 900-pound range cattle during normal consumption of the dry grass. The carotene content of this grass seems to be influenced by stage of maturity, temperature, precipitation, and many other factors, but there nevertheless appears to be an abundance of carotene, even through the winter months, to satisfy the vitamin A requirement of range cattle.

TABLE 1.—*Monthly carotene content of black grama and mesa dropseed grasses and related meteorological data*

[Milligrams of carotene per kilogram of feed, dry basis]

Date	Carotene [1] content of—		Average minimum monthly temperature	Total precipitation	Date	Carotene [1] content of—		Average minimum monthly temperature	Total precipitation
	Black grama	Mesa dropseed				Black grama	Mesa dropseed		
1937	*Milligrams*	*Milligrams*	° *F*	*Inches*	*1937*	*Milligrams*	*Milligrams*	° *F*	*Inches*
Feb 15	5.4	0	28.9	0.65	Nov. 15	80.0	0	30.5	--------
Mar 15	5.8	0	33.4	.44	Dec 15	26.6	0	29.0	0.48
Apr 15	[2]20.7	24.3	40.1	--------					
May 15	32.7	37.5	50.1	.87	*1938*				
June 15	82.8	81.7	57.9	.67					
July 15 [3]	52.5	93.7	65.1	.36	Jan 15	24.1	0	27.0	.55
Aug 15	38.2	84.7	65.5	1.04	Feb 15	15.7	0	31.4	.39
Sept 15 [3]	125.7	138.6	59.7	3.21	Mar 15	13.4	0	35.1	.06
Oct 15 [3]	105.9	68.4	46.2	.89	Apr. 15	[2]26.5	0	40.5	.03

[1] Carotene results are averages of duplicate determinations
[2] Average for April includes only 1 determination on black grama.
[3] Samples were ground in a mortar with sea sand.

The mesa dropseed grass during the growing season contained as much carotene, and in most cases a good deal more, than the black grama grass. However, the mesa dropseed grass dies with the coming of frost and freezing weather and the carotene is soon destroyed. In 1937 all the carotene was destroyed as early as November 15. This early loss of carotene suggests a somewhat limited winter usefulness of this grass except as a dry winter forage.

The seasonal characteristics of the two range grasses are clearly shown in figure 1. Although the amount of carotene in the black grama grass was slightly smaller than that in the dropseed grass during the growing season, the black grama was slower in maturing and maintained high carotene values for 30 days longer. The black grama always contained some carotene, even during the winter, when the amount was small, but no carotene was found in the dropseed from

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[6] GUILBERT, H R. See footnote 3.
——— MILLER, R. F., and HUGHES, E. H THE MINIMUM VITAMIN A AND CAROTENE REQUIREMENTS OF CATTLE, SHEEP, AND SWINE. Jour. Nutrition 13: 543–564. 1937.

November 15 to April 15. The high carotene period of both grasses occurred during the early part of the growing season, which was influenced to a large extent by the exact time of the summer rains.

Other workers also have found a rapid change in carotene content of grass with change in season. Atkeson, Peterson, and Aldous,[7] of the Kansas Agricultural Experiment Station, reported that typical pasture plants showed relatively high carotene values in early summer, marked decreases during the hot summer months, and high carotene values after the fall rains. Some of the native pasture grasses, such as big bluestem and buffalo grass, were very low in carotene content by November. Smith and Stanley,[8] of the Arizona Agricultural Experiment Station, working on the vitamin A potency of blue grama grass by the rat-growth method, found the early cut grass twice as potent as the mature grass and 100 times as potent as the November samples.

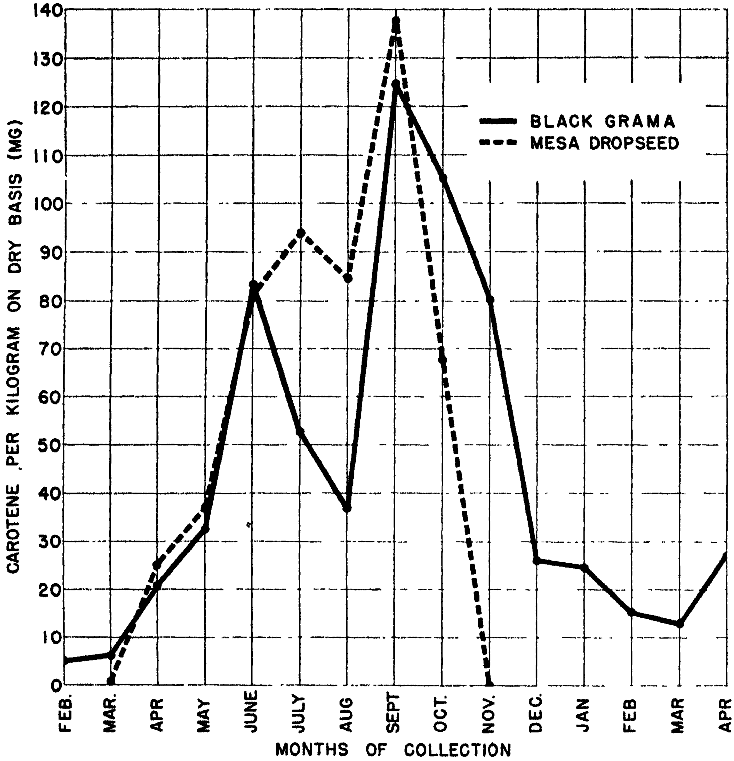

FIGURE 1.— Carotene content of two range grasses when samples were collected the 15th of each month.

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[7] ATKESON, F. W., PETERSON, W. J., and ALDOUS, A. E. THE OBSERVATIONS ON THE CAROTENE CONTENT OF SOME TYPICAL PASTURE PLANTS. Jour. Dairy Sci. 20: 557-562, illus. 1937.

[8] SMITH, MARGARET CAMMACK, and STANLEY, E. B. THE VITAMIN A VALUE OF BLUE GRAMA RANGE GRASS AT DIFFERENT STAGES OF GROWTH. Jour. Agr. Research 56: 69-71. 1938.

SUMMARY

The monthly carotene content of two important southern New Mexico range grasses, black grama and mesa dropseed, has been presented. Both grasses are moderately high in carotene during the growing season. The mesa dropseed loses all of its carotene soon after the fall freezes end the growing season. The black grama grass, whose upright stems remain green for a distance of from 4 to 6 inches of their base throughout the winter, contain an amount of carotene that seems to be ample to satisfy the vitamin A requirements of range cattle.

THE RELATION OF MOISTURE AND TEMPERATURE TO GROWTH OF THE COTTON ROOT ROT FUNGUS[1]

By C. H. Rogers

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INTRODUCTION

It is a matter of general observation by farmers and research workers that the spread of the cotton root rot fungus (*Phymatotrichum omnivorum* (Shear) Duggar) on cotton and other susceptible crops, and their resulting death, varies during the growing season to a rather large extent with available soil moisture. The disease spreads rapidly in hot weather following sufficient rainfall—that is, rains of about an inch or more. Taubenhaus and Dana (*8*)[2] analyzed air-temperature, rainfall, and air-humidity data as correlated with the percent of cotton plants killed by root rot during the years 1923, 1924, 1925, and 1926. They concluded that of the three factors, humidity has no direct influence; rainfall is outstanding in its importance; temperature for the crop-producing portion of the season is favorable to the development of the disease, and toward the end of the growing season lowered temperatures reduce root rot in severity in spite of favorable moisture. Taubenhaus and Ezekiel (*9*) reported a slow spread of the fungus from root to root throughout the winter to February 20 in two different fields near College Station, Tex.

To obtain a more direct relationship of moisture and temperature, especially the latter, to growth of the root-rot fungus, experiments were conducted under controlled conditions during the winters of 1931–32, 1932–33, and 1933–34. This paper gives the results of these studies.

METHODS

MOISTURE STUDIES

To determine the effect of moisture on the growth of the fungus and production of sclerotia, half-gallon size fruit jars were filled with soil of varying moisture content and inoculated with pieces of freshly infected cotton roots. The surface soil of Houston black clay was air-dried in the greenhouse for a few days, being turned at intervals to secure uniform drying. The moisture content of the air-dried soil was usually around 8 percent, oven-dry basis. In these experiments the cultures were set up so that soil moistures of 15, 20, 25, 30, 35, 40, and 45 percent, on the oven-dry basis, were obtained. These are equivalent to 21, 28, 35, 42, 49, 55, and 62 percent, respectively, of the maximum water-holding capacity as determined by the Hilgard method. Since water was mixed in an open container with the soil, a small amount was added in excess to take care of evaporation during the mixing process and while the soil was being transferred to jars.

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[1] Received for publication September 17, 1938. Contribution No. 477, Technical Series, Texas Agricultural Experiment Station.

[2] Italic numbers in parentheses refer to Literature Cited, p. 708.

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Samples were taken from each batch of soil to check the moisture content. All soil was rubbed through a quarter-inch mesh sieve while it was being mixed. After half a jar was filled with soil, five pieces of infected root inoculum were stuck vertically in the jar, and the remainder of the soil was added. In the case of higher moisture percentages, the soil moisture was made to about 25 percent by hand, and the remainder of the water added directly to the jar containing the soil.

Fungus growth was measured by methods described under Growth Measurements.

TEMPERATURE STUDIES

For making the temperature studies a water bath was constructed so that a series of nine different temperatures could be obtained at the same time. Sectional views of the apparatus are shown in figure 1. This equipment was constructed by making a single box of 20-gage galvanized iron, 126 inches long, and adding partitions of the same material so that nine compartments were formed, each 14 by 14 inches.

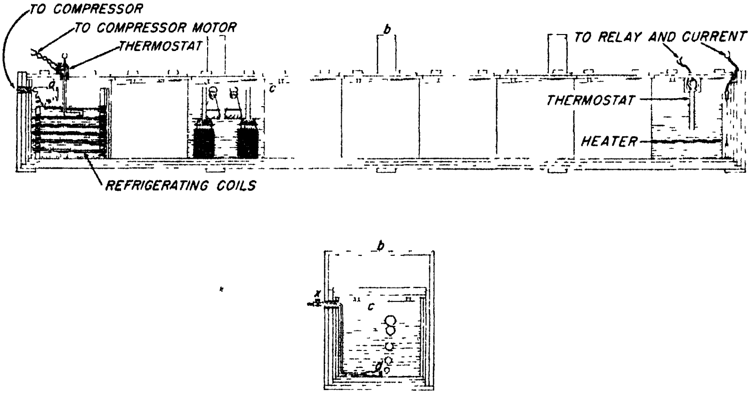

FIGURE 1.—Sectional views of water-bath apparatus used in making temperature studies.

Around this two layers of ½-inch Celotex were added and boxed in with 1-inch thick shiplap. Small slats were nailed on the bottom, around the sides, and across the top to support the box off the floor and to hold the shiplap against the Celotex (fig. 1, *b*). Covers for each compartment were made of two pieces of ½-inch Celotex held together by small slats. A piece of sheet iron was fastened at an angle on the under side of each cover so that water of condensation drained off to one side (fig. 1, *c*). Refrigerating coils were placed in one end of the box and connected with an ordinary household refrigerant compressor and motor assembly. Beginning with the expansion valve (fig. 1, *a*) coiling was begun at the top so that the coldest part of the coils was at the top of the water bath. Refrigeration was controlled by a bimetallic thermostat. In the opposite end high temperatures were obtained by housing the element from an electric oven in a copper box and connecting with the current through a relay of the type used on electric ovens and a pencil-type bimetallic thermostat. In this manner a series of different temperatures resulted across the nine compartments that varied with the change of the two thermostats. Agitation was obtained in each water bath by compressed air. An air line, made of copper tubing connected with an automatic air compressor, provided air for each compartment through an inlet of the same size tubing connected with heavy-walled rubber hose and controlled by a pinch clamp (fig. 1, *x*). A small boxlike piece of sheet iron (fig. 1, *d*) was soldered to the end of each inlet at the bottom of its respective compartment and tilted at a slight angle downward. In this way a large amount of air accumulated in the small box before a bubble escaped. As a result of this, the bubbles were large and adjustments were made so that they escaped at the rate of about one each 5 seconds. Pressure in the air line was kept at 15 pounds by a regulator to which a filter and gage were attached. During the course of each experiment the entire apparatus, shaded with opaque paper, was housed in the greenhouse thermostatically controlled at 65° F. The temperature in each compartment seldom varied more than 1° C. The apparatus as set up in the greenhouse is shown in figure 2.

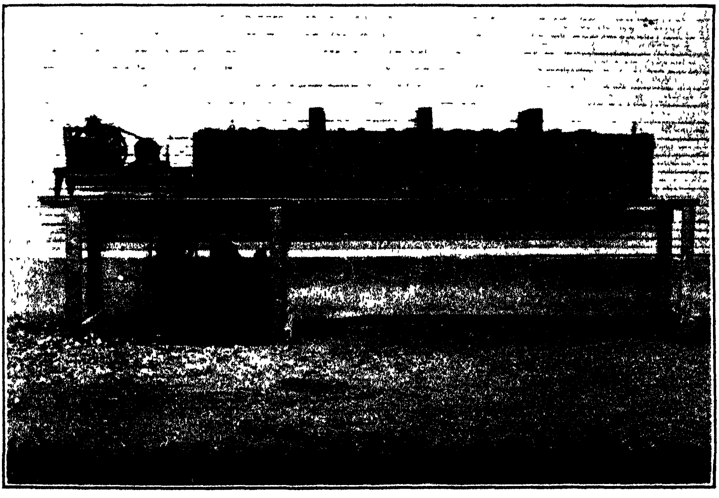

FIGURE 2.—Apparatus used for making temperature studies as set up in the greenhouse.

Growth responses were obtained in soil cultures in jars and in pure cultures on liquid media at the same time. Holes were punctured in the jar lids, and a 6-inch tube was fastened thereto so that it extended above the water line. A piece of hardware cloth was set on top of the jars, and the pure cultures in Erlenmeyer flasks were set on top of the hardware cloth. Each flask was weighted down with a lead ring. The jar cultures were made similar to those described under the moisture tests, and dry weights were obtained from liquid cultures made by using a medium constituted as follows: Water 1,000 cc, ammonium nitrate 1.2 g, potassium phosphate (secondary) 1.4 g, magnesium sulphate 0.8 g, potassium chloride 0.15 g, sucrose 30g, starch 30 g. Fifty cubic centimeters of this medium, which was made to pH 6.8, was used per flask and inoculated with small cubes of agar about 2 mm thick, containing young mycelial growth. Five jars and six flasks were used in each compartment for each experiment. At times *Rhizopus* or other fungi grew through and produced spores on the bottom of the cotton plugs. Such contamination was prevented by flaming the plugs and covering with sterilized sheet dental rubber.

GROWTH MEASUREMENTS

Whether *Phymatotrichum omnivorum* is growing superficially on culture media or along roots, compound mycelial strands are formed over the entire surface. These structures have been described previously (*1*, *2*, *6*). Toward maturity they assume a buff or brown color and may extend through the soil as much as 8 or 10 inches in either direction. The number and length of these strands as shown on the walls of the glass jars was used as one measurement of growth response. This applied only to the soil cultures in both moisture and temperature studies. Sclerotia are formed at intervals in strands by the compounding and compacting of hyphae. They are of great importance in making studies of cotton root rot because of their ability to remain in a viable state for many years and the fact that they may be formed in the soil at depths up to 4 feet or more. The number of sclerotia produced was used as another measurement of growth response in the soil-moisture and temperature cultures. Sclerotia were separated from the soil by methods previously described (*3*). In the pure cultures on liquid media growth was measured by obtaining dry weights of fungus, including the mat growing directly in and on the media and the strand mycelium and sclerotia growing on the flask walls.

RESULTS

SOIL-MOISTURE STUDIES

Production of strands and sclerotia in four different soil-moisture experiments are given in table 1. In the data the number and length of strands represent the average per jar in each individual test, set up in triplicate or quadruplicate for each different moisture percentage. These particular tests were conducted at different times during a 3-year period. The variation from test to test might therefore be caused by any one or all of three or four different factors, primarily differences in temperature from season to season and some differences in inoculum for the different years, or differences within the same year in age of plants, since in all cases cotton roots were used as the source of inoculum. Strands were produced in abundance in even the comparatively dry soil, diminishing rather sharply at and above 35-percent moisture content.

Sclerotia were produced somewhat sparingly even in those soils with optimum moisture for strand production. The optimum for both strand and sclerotial production seems to be from 25 to 30 percent. The number and length of strands is not always indicative of the growth in any culture. These strands branch rather freely in a dichotomous manner. Sometimes a single strand will cover two sides or more of a half-gallon jar. Over a number of cultures, however, the number and length of strands is indicative of the growth response under any given set of conditions. Sclerotia and strands in the drier cultures had a downy covering of hyphae of the acicular type. In the soils having higher moisture percentages, especially those nearing soil saturation, the strands were smoother and did not show much of the acicular-hyphae covering.

TABLE 1.—*Production of strands and sclerotia in soil cultures with quantities of water varying from 8 to 40 percent* [1]

Duration of experiment (days)		15 percent	20 percent	25 percent	30 percent	35 percent
5	Strands......number	1.0	3.0	4.3	0.7	1.3
	Strand length......centimeters	.8	.3	1.0	.3	.7
	Sclerotia......number	.0	.0	.0	.0	.0
24	Strands......do	9.8	5.8	12.0	8.5	2.5
	Strand length......centimeters	6.4	4.1	6.3	4.8	1.6
	Sclerotia......number	11.0	4.0	2.0	.0	.0
72	Strands......do	12.3	10.3	7.3	2.3	3
	Strand length......centimeters	8.3	7.3	8.8	14.3	.7
	Sclerotia......number	5.0	.0	10.0	91.0	0
120	Strands......do	1.7	6.0	8.7	4.7	6.3
	Strand length......centimeters	1.4	5.4	6.2	6.1	9.8
	Sclerotia......number	.0	.0	5.0	.0	.0

[1] No growth at 8-percent, air-dry, nor at 40-percent moisture.

TEMPERATURE STUDIES

The average weights of mycelial growth per 250-cc flask from four different temperature experiments are shown in tables 2 and 3. In the last two experiments (table 3) the heat-control thermostat was changed from 50° to 45° C.

TABLE 2.—*Average dry weight of root-rot fungus per 250-cc flask after 10 and 20 days growth at different temperatures from 3° to 50° C.* [1]

Growth period (days)	Dry weight when grown at—			
	19°	24°	29°	34°
	Milligrams	*Milligrams*	*Milligrams*	*Milligrams*
10	7	99	139	127
20	45	403	506	404

[1] No growth at 3°, 12°, 39°, 44°, or 50° C.

TABLE 3.—*Average dry weight of root-rot fungus per 250-cc flask after 10 and 60 days growth at different temperatures from 3° to 45° C.* [1]

Growth period (days)	Dry weight when grown at—					
	11°	18°	23°	27°	32°	37°
	Milligrams	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
10	0	6	27	170	218	4
60	30	336	492	830	390	15

[1] No growth at 3°, 42°, or 45° C.

The optimum range for fungus growth is from approximately 23° to 34° C., the middle of this range being 27° to 29°, as shown by the experimental results. In the first experiment an average of 506 mg of fungus was obtained after a 20-day growth period at 29°. In the last experiment at the end of 60 days an average of 830 mg was obtained at 27°, these figures representing the maximum growth in each experiment. At all times in the 3° bath there was a rim of ice on the coils that almost reached the center of the compartment. Therefore the flasks in this compartment were surrounded by ice at least part of the time. Hyphae in the flasks in this coldest chamber grew about 2 mm during the first 2 days but stopped thereafter, and the tips seemed to curl backward in a somewhat hooked manner. Although there was no growth under such low-temperature conditions, the fungus readily resumed growth when transferred to warmer baths at 27° or 29° even at the end of the longest period of 60 days. At the higher temperatures the effect was directly opposite. At 39° or above no growth was obtained, and when cultures were transferred to 27° or 29° baths there was no resumption of growth, even at the end of the shortest experimental period of 10 days. This indicates that all fungus growth had been killed.

Microscopic examination of mycelial growth showed no general morphological differences. There was one striking difference in appearance. At the lower temperatures up to 19° C. the mycelium remained almost white, never getting darker than a light amber color. With increase in temperature the entire mycelium showed a darker color regardless of the amount of growth. The same thing held true in the case of the soil cultures at different temperatures. Both strands and sclerotia were formed with the light or whitish color that the mycelium usually exhibits in the younger stages of growth, retaining this light color throughout the experimental period. Throughout the optimum-growth range the fungus exhibited characteristics typical of those observed under field or ordinary laboratory conditions—that is, at first there was a whitish web which gradually increased in size and became darker until the brown or buff color of maturity was attained. At the higher temperatures, at and above 34°, the mycelium was first formed with the dark color of maturity and retained this or became darker throughout each growth period Color, therefore, is not necessarily an indicator of age of sclerotia, as is generally thought to be the case. In field areas almost all light-colored sclerotia are found in the late fall or early winter after cool weather has begun.

The number of sclerotia produced at different temperatures after 30-, 60-, and 80-day growth periods are shown in table 4. Each of these different periods represents a separate experiment, all the jars in each chamber being analyzed for sclerotia for each separate experiment. Where sclerotia are formed under such conditions they are usually produced within the first 30 days and certainly none after 80 days, because the inoculum or substratum has been depleted and decomposition has set in. Optimum temperature for sclerotial formation is somewhat the same as that for general mycelial growth, although in the 80-day experiment the greatest abundance of sclerotia was produced at 18° C. The greatest amount of strand formation and sclerotial production was obtained at 27°.

TABLE 4.—*Production of root-rot sclerotia in soil cultures at different temperatures from 3° to 45°C. after 30, 60, and 80 days*[1]

Growth period (days)	Number of sclerotia when grown at—					
	11°	18°	23°	27°	32°	37°
30	0.0	0.0	95.0	207.0	29.0	0.0
60	.0	.0	.0	209.0	8.0	52.0
80	66.2	293.8	131.6	147.2	12.0	8.8

[1] No growth at 3°, 42°, or 45° C

DISCUSSION

As pointed out by Taubenhaus and Dana (*8*) and as commonly observed, moisture is the main limiting factor for root-rot spread during the growing season. The temperature is sufficiently high from May through October for ready growth of *Phymatotrichum omnivorum*. Usually by the first of May the ground is sufficiently warm for rapid spread of the fungus, if host material is present in a fairly dense stand. After rains in July, August, and September there is usually a rapid spread of root rot in cottonfields as evidenced by the wilting and speedy death of the cotton plants. In years of low soil moisture throughout the growing season root rot is held in check. In such years there may be a general kill of 25 percent, whereas in years of intermittent rainfall throughout the growing season the amount of cotton killed may be as high as 50 percent. Early or midseason killing of cotton plants results in low yields (*5*).

These moisture and temperature experiments and general field observations indicate that *Phymatotrichum omnivorum* thrives over a rather wide range of moisture and temperature conditions. The fungus is, however, readily affected by high temperatures and low moisture conditions. This accounts for the fact that no mycelial strands and sclerotia are found in the upper 6 inches of soil except in periods of warm wet weather. At such times the white mycelium may be found or seen growing as a collar around the stalks of cotton or other host plant just at the ground line. It is also during these periods that the mycelium grows to the surface and produces the downy conidial mats which may vary from the size of a dime to 18 inches or more in diameter. These structures are formed in the shade of low-growing field crops or sometimes under bushes or trees where the soil is not subject to the drying effect of the sun's rays and remains moist for a few days. Sclerotia have been found thus far to remain in a viable condition for 8 years in the soil (*4, p. 204; 7*). If these bodies are left exposed in the open, they lose their viability within a very short period, usually less than an hour, depending on air temperature and humidity. There is a decided difference in this respect between the sclerotia of *Phymatotrichum omnivorum* and those produced by certain other fungi. The mycelial strands also rapidly lose their viability under the same conditions.

Taubenhaus and Ezekiel (*9*) found that there was a spread of root rot along the cotton roots throughout the winter near College Station, Tex. This district is near the lower end of the blackland section, the section where cotton root rot is worst, but it is quite probable that such spread occurs in all parts of the blackland. Soil temperature records taken at the Temple Substation at depths of from 2 to 48 inches show that at the 18-inch depth or deeper the temperatures are never too cold for growth of *Phymatotrichum omnivorum*. There is no doubt, however, that growth is slowed down greatly during the winter. Host material is the main limiting factor in the spread of the fungus. Active root rot has not been found earlier than April on winter annual weeds. The growth reported by Taubenhaus and Ezekiel was on old cotton plants growing in the field from the previous season. It is necessary that cotton plants become fairly well established before attacks by the fungus occur, since no seedlings have been found dying from the cotton root rot disease. This is partially owing to the fact that there is little or no mycelial growth in the upper layers of the soil.

SUMMARY

Mycelial strands of the cotton root rot fungus (*Phymatotrichum omnivorum* (Shear) Duggar) grew in Houston black clay soil in which the soil moisture varied from 15 to 35 percent, oven-dry basis. The optimum moisture content was around 25 percent, which is equivalent to 35 percent of the maximum water-holding capacity of the soil. Sclerotia of *P. omnivorum* were produced at a soil moisture content of from 15 to 30 percent. Neither sclerotia nor mycelial strands grew at or below 8 percent or above 35 percent soil moisture.

Both mycelial strands and sclerotia were produced in constant-temperature water baths at temperatures of from 11° to 37° C., the optimum for both being approximately 27°.

At the lowest experimental temperature of 3°, there was no active growth, but apparently the fungus was not injured. Temperatures of 39° and above resulted in the death of the fungus.

At the lower temperatures both the sclerotia and mycelium formed were of a whitish-amber color. At the minimum temperature at which growth occurred this light color was retained during the entire experimental period.

At higher temperatures both sclerotia and mycelium were formed with the usual dark color of maturity, as found in specimens taken from field soil.

Both mycelial strands and sclerotia were rapidly killed by exposure to high temperatures or to drying, such as takes place under ordinary field conditions in the summer.

Temperatures throughout the crop-growing season are favorable for growth and spread of *Phymatotrichum omnivorum*. During this period, moisture is the limiting factor, intermittent rains of sufficient quantities causing rapid spread of the fungus, a large proportion of the cotton plants being killed by the end of the season.

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BILATERAL VARIATION IN THE WEIGHT AND COMPOSITION OF THE LONG BONES OF SOME EXPERIMENTAL ANIMALS[1]

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INTRODUCTION

The literature dealing with bilateral variation in the bones of experimental animals and suitable for close comparisons is meager and scattered. Investigators have sometimes used bones from one side of an animal for one type of determination and the corresponding bones from the other side for a different type of determination, with the tacit assumption that the bones from the two sides were identical in composition or other properties. Some years ago an investigation was begun at this laboratory to determine the composition of various skeletal parts of experimental animals. This afforded an opportunity for a study of bilateral variation in the long bones.

MATERIALS AND METHODS

In the study herein reported four kinds of animals were under experiment, viz, dairy cattle, swine, chickens, and rats. In all cases the animals were on feeding trials designed to produce differences in bone development; hence the data include bilateral comparisons in individuals on both normal and deficient rations. In order to obtain as accurate a representation as possible of the bone in its natural state and to avoid variations in sampling, whole green bones were used for analysis throughout. In certain instances two or more bones were combined to form a single composite sample. The bones taken for study from each type of animal were as follows:

Rat: Femur, tibia, and fibula (composite); humerus, ulna, and radius (composite). Total of four samples.

Chicken: Femur; humerus; tibia; ulna and radius (composite). Total of eight samples.

Swine: Femur; humerus; tibia; ulna and radius (composite); seventh and eighth ribs (composite). Total of 10 samples.

Dairy cow: Femur; humerus; tibia; metacarpus; metatarsus; ulna and radius (composite); seventh and eighth ribs (composite); thirteenth rib. Total of 16 samples.

The bones were removed from the fresh carcass, freed from adhering tissue, and weighed. In dissecting out the bones all muscle, tendons, and ligaments were carefully removed. The periosteum and articular cartilages, however, were left intact on the shaft and so were included in all samples.

The swine and cattle bones were ground first in a power-driven Mann bone grinder and immediately thereafter in a small hand-operated food chopper. The few pieces failing to pass through the grinding machine were carefully collected and reduced by hand in a

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covered iron mortar. After drying in vacuo and extracting in large Soxhlet extractors, the resulting moisture-free, fat-free material was ground finely in a Wylie mill and the ash determined.

The chick and rat bones were crushed between the jaws of pliers, cut into small lengths with diagonal cutting pliers, dried in vacuo, extracted in a modified Bailey-Walker extraction apparatus,[2] and ashed in fritted-bottom Jena glass crucibles without transfer after preparation for drying.

The determinations made on all bones were green weight, moisture, ether extract, ash, and organic nonfat (by difference).

Since the determinations made on each animal were paired throughout as lefts and rights, the differences found were studied statistically by Student's method and compared according to Livermore's table of odds [3] as shown in table 2.

The 20 rats used were albinos—10 males and 10 females; 5 of each sex were on a normal ration and 5 on a rachitic ration. The rats were killed at 65 and 70 days of age.

The 20 chicks used were White Leghorns—10 males and 10 females; 5 of each sex were on a normal ration and 5 on a rachitic ration. The bones were taken at 35 days of age.

The 19 hogs included 8 Poland China, 4 Duroc Jersey, 3 Berkshire, and 4 cross-bred animals. There were 14 males and 5 females. The animals ranged in age from 193 to 288 days at time of slaughter.

Two grade Holstein heifers supplied the bones for the comparisons with dairy cattle. One was fed a normal ration and the other was fed a ration low in phosphorus. After dissection from the carcass, all bones were broken open with a cleaver and the marrows were separated and removed as completely as was practicable from all except the ribs. Marrows and bones exclusive of marrows were weighed and analyzed separately. The analytical results were combined later to represent the values for whole bones. The animals were 15 months old at time of slaughter.

EXPERIMENTAL RESULTS

The average values for the various bones and determinations are shown in table 1. An examination of this table reveals some slight difference between the left and right fore and rear composites in the rat, but a statistical examination of these differences shows them to be without significance for 9 of the 10 comparisons made, the exception being ether extract in the tibia-fibula-femur composite sample. In 1931 Jones and Robson[4] reported separately the ash content of right and left femurs from 32 adult rats. From their data the average percentage of ash may be calculated as 62.89 for the right and 62.90 for the left femur, respectively, in the dry, extracted bone. Hume, Pickersgill, and Gaffikin [5] also reported the ash content of right and left composite samples of tibia and femur for 88 individual rats, and a calculation of the average percentage of ash in dry extracted bone from their data yields identical values of 44.28 for either right or left. In the present investigation the ulna and radius were combined with the humerus, and the tibia and fibula were combined with the femur on either side. The bones from the left foreleg average 0.3 percent higher in ash (green basis) than those from the right foreleg, but the bones from the right rear leg are higher than those from the left by a like amount. It seems, therefore, that insofar as comparisons can be made, these data are in agreement with those of other investigators in showing that in the rat there is no significant difference between the ash content of long bones from the right and left sides. Nor is there any significant difference in weight of green bone, percentage of moisture, or organic nonfat in the bones from the two sides. In the rear leg the ether extract is significantly higher in the bones from the left side, but in the foreleg the trend is toward higher values on the right side. When the values for fore and rear legs are combined the difference between left and right is without significance.

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TABLE 1.—*Average weight and composition of rat, chicken, swine, and dairy-heifer bones on green basis*

Bone	Green weight		Moisture		Ether extract		Ash		Organic nonfat	
	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right
	Grams	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
RAT										
Ulna, radius, and humerus	0 2541	0 2543	47 03	47 07	2 05	2.17	25 77	25.47	25 15	25 29
Tibia, fibula, and femur	6134	6093	50 15	49 91	3 91	3 77	22 27	22 58	23 67	23 74
Ulna, radius	0.7016	0 6934	56 73	56 04	7 64	7 86	14 70	14 91	20 93	21 19
Humerus	9718	9340	61 99	61 19	4 23	4 00	13 17	13 89	20 61	20 92
Femur	1 2021	1 2119	60 79	60.54	4 30	4 44	13 95	14 12	20 96	20 90
Tibia	1 5594	1 5166	60 02	59 62	5 84	5 83	14 43	14 68	19 71	19 87
SWINE										
Ulna, radius	182 94	181.80	26.27	26 17	15.02	15.33	36.37	36.28	22.34	22.22
Humerus	254 43	252 40	27 76	27 58	18.59	18.93	33.29	33 39	20.36	20 10
Femur	276 12	274.35	27.90	27.53	20 43	20.49	32.13	32 44	19.55	19 54
Tibia	171.21	169 77	25.46	24 93	17 51	17 75	35.70	36.06	21.34	21 27
Ribs, seventh and eighth	67.64	66 83	30.59	30 54	10.63	10.42	35 72	36.12	23 07	22 92
DAIRY HEIFER										
Metacarpus	280.5	291 5	24.18	25 82	24 65	23.24	31 12	30 75	20 05	20 19
Ulna, radius	657.8	673.7	27.61	27.61	26 50	26.83	26 71	26 51	19 18	19 05
Humerus	858.9	862.2	33 44	33 27	26 75	27 53	21.83	21 75	17 98	17.45
Femur	1,196 2	1,206.0	34.60	36 50	28.17	25 38	20 63	20 65	16.60	17.47
Tibia	765.6	790.7	28.20	29.62	26.57	23.94	26 87	27 63	18 36	18.81
Metatarsus	342.0	351.2	21.21	21 95	26.73	25.18	32.05	32 10	20 01	20 77
Ribs, seventh and eighth	286.8	294.5	46.89	43 83	7.51	10.01	23.34	23.81	22 26	22 35
Rib, thirteenth	51.3	51.1	40.39	38.56	4.98	5.67	29.26	29.92	25.37	25 85

Turning next to a consideration of the data from chick bones (table 1), it is apparent that a somewhat different situation exists. For all bones analyzed, except the femur, the average weight is greater on the left side, as is percentage of moisture in all instances, while percentage of ash is greater on the right side. Apparently green weight and moisture content tend to run higher on the left side, whereas ash varies inversely with moisture and is higher for the bones of the right side. Table 2 shows the significance of the differences between the two sides for individual bones and for combined values. For combined values the odds are significant for all determinations made on chick bones except ether extract.

TABLE 2.—*Approximate odds that bones from one side exceed those from the other side with respect to a given determination*

Animal and bone	Analyses on each side	Green weight	Moisture	Ether extract	Ash	Organic nonfat
Rat:	*Number*	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*
Ulna, radius, humerus	20	R>L 1:1	R>L 1:1	R>L 5.1	L>R 4:1	R>L 3:1
Tibia, fibula, femur	20	L>R 3:1	L>R 5:1	L>R 55:1	R>L 9.1	R>L 3:1
Chick:						
Ulna, radius	20	L>R 4.1	L>R 325:1	R>L 16:1	R>L 6:1	R>L 32:1
Humerus	20	L>R 500.1	L>R 269:1	L>R 2:1	R>L 3,200:1	R>L 21.1
Femur	20	R>L 4.1	L>R 18.1	R>L 7:1	R>L 14:1	L>R 3:1
Tibia	20	L>R 240:1	L>R 18:1	L>R 1.1	R>L 12.1	R>L 17:1
Swine:						
Ulna, radius	19	L>R 7:1	L>R 3:1	R>L 15:1	L>R 2:1	L>R 11:1
Humerus	19	L>R 32:1	L>R 7:1	R>L 18:1	R>L 3:1	L>R 26:1
Femur	19	L>R 11:1	L>R 49:1	R>L 2.1	R>L 2:1	L>R 1:1
Tibia	19	L>R 15:1	L>R 624:1	R>L 4:1	R>L 22:1	L>R 3:1
Ribs, seventh and eighth	19	L>R 9:1	L>R 2:1	L>R 8:1	R>L 26:1	L>R 26:1
Combined values for all bones of—						
Rat[1]	40	L>R 2:1	L>R 2:1	L>R 1:1	R>L 1:1	R>L 4:1
Chick	80	L>R 500:1	L>R 10,000:1	R>L 4:1	R>L 3,000:1	R>L 115:1
Swine	95	L>R 1,000:1	L>R 1,000:1	R>L 14:1	R>L 300:1	L>R 250:1
Dairy heifer[2]	16	R>L 121:1	R>L 1:1	L>R 4:1	R>L 7:1	R>L 25:1

[1] If weights from an additional series of 19 rats killed 2 years prior to those given in this table are included, the total number of bones weighed from each side becomes 78 and the odds for weight become 3:1 that L>R.

[2] Since bones were analyzed from only 2 dairy animals the odds for individual bones are omitted.

The swine bones (table 1) show relationships in general agreement with those set forth for the chick bones. Without exception the average values for green weight and moisture content are higher on the left side than on the right, while in all instances but one the percentage of ash is higher in the bones of the right side. Table 2 shows that here, also, the odds for combined values are significant for all determinations except ether extract. The direct relation between green weight and moisture content and the inverse relation between moisture and ash content may be shown by constructing simple charts or scatter diagrams (not included herewith) setting forth the individual instances in which both determinations run higher on the same side and the corresponding instances in which the higher values are found on opposite sides. If this is done for the 95 pairs of hog bones it will be found that in 62 of the comparisons the percentage of moisture varies directly with green weight, while in 71 of the comparisons the higher percentage of ash is on the side opposite the higher percentage of moisture. The charts will show further that no single bone or group of bones is responsible for this relationship or for the dominance of one side over the other, but that all bones share in the relationship. Breed and sex may likewise be shown to be without pronounced effect.

If we designate as 5–L an animal in which all five bones on the left side are heavier than the corresponding bones on the right side, as 4–L an animal in which all four bones on the left side are heaver than the corresponding bones on the right, etc., a table may be constructed showing the relationships of breed to dominance in weight. Table 3 gives such data.

TABLE 3.—*Tendency by breeds of swine toward dominance of one side over the other side in weight of green bone*

Breed	Individuals in each group [1]					
	5-L	4-L	3-L	3-R	4-R	5-R
	Number	*Number*	*Number*	*Number*		
Poland China	1	2	3	2	None	None
Duroc Jersey	1	2	0	1	...do...	Do.
Berkshire	0	1	0	2	...do...	Do.
Cross-bred	0	1	2	1	...do...	Do.
Total	2	6	5	6	0	0

[1] 5-L indicates an animal with all 5 bones on the left side heavier than the corresponding bones on the right side, 4-L one with 4 and 3-L one with 3 bones on the left side heavier than the corresponding bones on the right side. 3-R indicates an animal with 3 bones on the right side heavier than those on the left.

From these figures it appears that in the 19 hogs studied there were no strongly right-dominant individuals, but there were a number of strongly left-dominant ones. The data indicate further that no single breed is chargeable with the tendency toward heavier bones on the left side, but that all seem to contribute to this end. On the other hand, at least one individual of each breed and one cross-bred animal showed moderate right dominance.

The number of dairy animals available for study was too small to be of any considerable value in establishing clear-cut tendencies in bilateral comparisons. However, the average values presented in table 1 for the two heifers studied show an unmistakable trend toward heavier bones on the right side and a direct relation of moisture to green weight in 12 of the 16 comparisons, while the inverse relation of ash to moisture holds for 11 of the comparisons as shown by scatter diagrams (not included herewith). The odds for right or left dominance in moisture and ash are not significant, but odds for right dominance in organic nonfat approach significance.

The organic nonfat fractions of the chick and hog bones afford an interesting and somewhat puzzling comparison. The combined values in table 2 show that in swine organic nonfat is significantly higher on the left side along with green weight and percentage of moisture. In the chick, on the other hand, organic nonfat is significantly higher on the right side although green weight and moisture are higher on the left side as in the hog bones.

A study of comparative weights (rather than percentage) of moisture, ether extract, ash, and organic nonfat in the corresponding left and right bones from chicks and swine shows that where an appreciable difference in total weight of green bone exists between the two sides, this difference is likely to be chiefly in the moisture fraction, although the other constituents also may contribute to the difference in total weight.

SUMMARY AND CONCLUSIONS

In a study of bilateral variation in the weight and composition of green bones of 20 rats, 20 chicks, 19 hogs, and 2 dairy heifers the following results were obtained:

(1) No significant differences in weight of green bone or in percentage of moisture, ash, and organic nonfat were found in bones from the right and left sides of the rat.

(2) In the chick bones green weight and percentage of moisture were significantly higher on the left side while percentage of ash and organic nonfat were significantly higher on the right side.

(3) In the hog bones green weight, percentage of moisture, and percentage of organic nonfat were significantly higher on the left side while percentage of ash was significantly higher on the right side.

(4) The dairy heifers showed a tendency toward heavier bones on the right side.

(5) In animals in which one side showed a tendency to dominate in weight of bone the same side tended to be higher in percentage of moisture and the opposite side to be higher in percentage of ash.

These results may be interpreted as suggesting (1) that certain species of lower animals may tend toward a dominance of one side over the other with respect to bone development, and (2) that bones from one side are not necessarily identical in composition or other properties with those from the other side, although in general the differences found in these experiments were quite small.

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OPTIMUM SOLUTIONS AS PHYSIOLOGICAL REFERENCE STANDARDS IN ESTIMATING NITROGEN UTILIZATION BY ASPERGILLUS NIGER[1]

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INTRODUCTION

The absence of biological reference standards, it is believed, has contributed to the difficulties in the physiological interpretation of the quantitative data of mineral nutrition. Though accurate control of the physical environment in laboratory studies with organisms has become frequent, the use of nutrient solutions physiologically indeterminate is still a routine procedure. Comparisons between the quantitative responses of organisms under these conditions would seem of lessened value since only one of the innumerable solutions that can be employed provides an accurate scientific basis for comparisons. This is the optimum nutrient solution for growth containing a minimum quantity of constituents (*12*),[2] and is the only one whose composition is subject to exact definition on the basis of physiological response. It is the nutrient solution whose every component serves most efficiently in the production of mass by the organism within a precisely defined physical environment.

The advantages to be derived from the use of optimum solutions will be readily apparent to the physiologist. The effects of nutrients under these conditions are no longer determined on the basis of absolute values, but become comparative and relative to the standard physiological effects produced at their optimum concentrations. Since the effects of a variation will always be relative to those obtained under standard conditions (optimum), they should be directly comparable. The same advantage should hold for comparisons between different organisms. Whether the results obtained at one level of carbohydrate nutrition are strictly comparable to those holding at another level with the same organism is an important consideration, however. Complete agreement between results at different levels of carbon nutrition would enhance the value of all data inasmuch as it would indicate their general significance in the interpretation of the organism-solution system.

This procedure has been employed in this brief study of the nitrogen nutrition of *Aspergillus niger* Van Tiegh. It was considered that the extent of agreement in results relative to the respective maxima of the optimum solutions would furnish an adequate test. The accumulation of experimental results, therefore, began with the study of the optima for the known mineral constituents in an ammonium nitrate solution at a practically optimum temperature, at three levels of carbohydrate nutrition. The effects of nitrogen concentration on yield, acidity of the culture solution, and sporulation were then obtained with each of these solutions. An analysis was made of the mineral constituents in the mycelial felts grown on the three optimum solutions; but only nitrogen was determined in the material obtained by variation of the nitrogen supply. Comparative experiments were performed with ammonium nitrogen and with nitrate nitrogen, except that analyses were omitted.

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[2] Italic numbers in parentheses refer to Literature Cited, p. 731.

Brief reference, in addition, is made to studies on the theoretical equation for growth. The value of empirical growth equations for computing fertilizer requirements of plants has been recognized by Russell (*11*). Ojima (*8*) was able to compute the quantities of mineral nutrients absorbed. Special emphasis will be placed on the Mitscherlich growth formula (*11*) because of the attempts made to assign physiological meaning to its constants. Much of the discussion relating to this formula might have been avoided if it were possible to grow the higher plants so as to obtain yield data of high precision. Even under controlled environmental conditions (*14*), however, it is impracticable to obtain the necessary precision, because of the limited number of plants that can be grown in an artificial environment. The increased precision of control practicable with fungi has made possible an improvement in accuracy of physiological results.

Considerable space, therefore, has been devoted to the tabulation of data and the discussion of experimental precision as an important phase of general interest. Control of the physical environment and elimination of individual differences by the use of average values are responsible, no doubt, for much of the accuracy attained. An equally important contribution to precision has been made through control of the chemical environment. Purity of reagents is indispensable, of course, but it is equally important that each essential element be present in physiologically measured amount. Even though yields are maximum it is no exaggeration to assume that each essential element that participates in the development of the organism unknown to the investigator contributes directly to experimental inaccuracy, since the presence of each of these elements depends on impurities in the chemical compounds. Reliance on chance additions necessarily implies the use of fluctuating concentrations. It is doubtful, therefore, that the limit in precision has been reached, since it is almost certain that not all the chemical elements essential for the nutrition of *Aspergillus* are known.

Though the factors underlying the distribution of nutrients between organism and nutrient solution have received considerable attention, an adequate explanation of all phases of absorption has not as yet been accomplished (*7*). The ability of an organism to absorb a nutrient against a concentration gradient has been of special interest to investigators. Data are submitted, therefore, which indicate that relative solubilities of a compound in protoplast and nutrient solution may afford a partial explanation supplementing that given by the Donnan equilibrium. Computations of total nitrogen in the mycelial felts were made with considerable accuracy on the basis of an assumed constant protoplasmic nitrogen content and the distribution of the remainder between organism and solution according to laws governing the distribution of a solute between two immiscible solvents.

No attempt will be made to review the literature bearing on the mathematical formulation of growth, in view of the detailed and thorough treatment of this subject by Russell (*11*) recently. Other citations will be mentioned in connection with the material to which they have reference. There is no experimental material available for a study of the effects on the growth of an organism of a graduated variation in a mineral constituent as compared with an optimum solution of minimum salt content.

METHODS

The cultural methods employed with *Aspergillus niger* have been given in previous publications (*13*) and need not be repeated except to state that the fungus was grown for 4 days at 35° C. in 200-cc flasks containing 50 cc of nutrient solution. Additional necessary information is furnished in connection with the experimental data.

Chemical determinations were made by means of the official methods for ash (*1, p. 336, par. 8*), nitrogen (*1, p. 137, par. 53*), phosphorus (*1, p. 130, par. 306*) magnesium (*1, p. 124, par. 12*), and sulphur (*1, p. 129, pars. 28–29*). Hicks' (*4*) method was used for potassium after preparation of the sample by the method of West (*15*).

Only single determinations were made for total nitrogen inasmuch as many of the samples (combined mycelial felts of duplicate cultures) provided very little material for analysis. The determinations listed in table 3, however, were made in duplicate with material grown in 2-liter flasks.

OPTIMUM NUTRIENT SOLUTIONS

The plan employed in this study is based on the use of optimum solutions containing the minimum quantities of inorganic constituents sufficient for maximum growth of the fungus with 50 cc of nutrient solution for 4 days at 35° C. These solutions were developed from suboptimal solutions by successively adding small increments of such components as had proved deficient in quantity in the preceding test, until the proper concentrations had been determined. The number of successive trials necessarily varied with each of the five solutions but usually more than four trials were required. Only when further addition of a small quantity of each component of the nutrient solution failed to bring about an increase in yield were the solutions considered suitable for studying the effects of a variation of nitrogen on yield. The reverse statement, that a slight decrease in any constituent will result in a diminished yield, is also true, of course. The composition of the nutrient solutions thus developed is given in table 1. The salt requirements of the fungus with variation in concentration of sucrose seem to exactly parallel the concentration of sucrose at these levels, if due allowance is made for deviations due to uncontrollable variations in impurities. The lesser need for minerals with ammonium chloride than with sodium nitrate is correlated, in all probability, with the higher acidities developed in cultures with the former as a consequence of relative accumulation of the chloride ion.

TABLE 1.—*Composition per liter of optimum nutrient solutions* [1]

Constituent	Composition of solution having as source of nitrogen—				
	NH_4NO_3			NH_4Cl	$NaNO_3$
	Grams	*Grams*	*Grams*	*Grams*	*Grams*
Sucrose	25 0	50 0	75.0	50 0	50 0
NH_4NO_3, NH_4Cl, or $NaNO_3$	.88 (84)	1 90	3 05	2 54	4.04
K_2HPO_4	17	35	60	42 (35)	.42 (.35)
$MgSO_4.7H_2O$	10	.25	.53	30 (.25)	30 (.25)
Fe	00008 (7)	.00020	.00036	00020	.00024
Zn	00011 (10)	.00020	.00032	00020	00030
Cu	.00001	00005	.00009	.00005	.00008
Mn	00001	00002	.00005	00002	.00005
Mo	00001	00002	00003	00002	00006

[1] The values in parentheses are those actually used in the experiments of table 2, where they differed from those of the solutions used in determining the nitrogen curves and obtaining materiel for chemical analysis.

Table 2 shows growth data obtained with the five optimum solutions. The percentages of maximum yield with the trace elements indicate the extent of these elements present as impurities in the solutions. The yields obtained by increasing the individual constituents are a measure of their deficiency, if any, in these solutions. Even casual examination will disclose that these solutions are practically optimum for growth. The yields, moreover, are proportional to the quantity of sugar and independent of the source of nitrogen. The yields per unit quantity of sugar are corrected values and not those actually obtained. In the preparation of the solutions the total volume called for is usually exceeded slightly. Correction for the excess has been estimated experimentally to be equivalent to 0.002 times the excess in cubic centimeters per liter. This value in milligrams is added to the yield per culture if the nutrient solution was too dilute, and if, as occasionally happens, the solution was too concentrated, it is substracted.

The values for certain of the mineral constituents of the mycelial felts grown in the three optimum solutions with ammonium nitrate are given in table 3 for each of the three sucrose concentrations studied. Several interesting features appear. The percentage contents of the different constituents in the mycelial felts are, on the whole, unusually low (*9*) and vary but little in the three solutions. The deviation from the mean of the three experiments is less than ±5.0 percent for ash, nitrogen, phosphorus, and sulphur; ±7.9 percent for potassium, and ±10.5 percent for magnesium. The accuracy with which yields could be determined with certainty was but slightly better, however. Practically no difference was found between the percentages of utilization of the nutrient components at the three sucrose levels. In other words the analytical results with the mycelial felts grown in the three optimum solutions were practically identical with respect to percentages of mineral constituents in the felts and the degree to which these constituents were absorbed. Variation in nonprotoplasmic mineral constituents resulting from passive absorption readily accounts for any slight differences that occur.

TABLE 2.—*Growth phenomena with Aspergillus niger when grown in optimum solutions for 4 days at 35° C.*

Component omitted from (−) or added in excess to (+) the nutrient solution	NH_4NO_3 (2.5 percent of sucrose)				NH_4NO_3 (5.0 percent of sucrose)				NH_4NO_3 (7.5 percent of sucrose)				NH_4Cl (5.0 percent of sucrose)				$NaNO_3$ (5.0 percent of sucrose)			
	Yield per 1.25 g sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation[1]	Yield per 2.5 g sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation[1]	Yield per 3.75 g sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation[1]	Yield per 2.5 g sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation[1]	Yield per 2.5 g sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation[1]
	Mg	*Percent*	*pH*		*Mg*	*Percent*	*pH*		*Mg*	*Percent*	*pH*		*Mg*	*Percent*	*pH*		*Mg*	*Percent*	*pH*	
None	534.2	100.00	2.21	10, br	1,152.0	100.00	2.37	9, bl	1,658.6	100.00	2.76	10, bl	1,009.2	100.00	1.46	6, bl	1,002.8	100.00	2.81	10, bl
−Fe	104.6	19.58	2.25	1, bl	173.8	15.08	2.12	3, bl	322.2	19.43	1.98	4, bl	484.7	48.03	1.67	4, bl	207.3	20.67	3.25	8, bl
−Zn	35.5	6.64	2.76	2, bl	106.6	9.25	2.46	6, bl	172.7	10.41	2.33	4, bl	44.9	4.45	1.93	4, bl	211.1	21.05	3.16	8, bl
−Cu	504.9	94.51	3.06	4, t	1,011.6	87.81	3.15	1, bl	1,449.4	87.39	3.65	2, w	950.9	94.22	1.44	0	893.8	89.13	3.86	6, t
−Mn	431.7	80.85	1.82	0	899.7	78.10	1.68	1, bl	1,370.3	82.62	1.72	2, bl	778.5	77.14	1.35	1, bl	922.9	92.03	2.11	6, bl
−Mo	380.5	71.23	2.16	2, bl	706.3	61.30	1.82	1, bl	1,082.6	65.27	1.67	2, bl	1,012.2	100.30	1.42	3, bl	108.7	10.84	3.30	6, bl
+Fe	558.9	104.61		10, br	1,203.0	104.42		9, bl	1,646.0	98.07		10, bl	984.9	105.60		4, bl	1,013.5	101.08		10, bl
+Zn	562.8	105.35		10, br	1,162.6	100.92		9, bl	1,630.2	97.13		9, bl	988.8	97.98		4, bl	1,038.1	103.53		10, bl
+Cu	529.0	99.02		10, bl	1,100.8	95.35		9, bl	1,691.5	101.98		10, bl	1,005.9	99.68		4, bl	1,036.2	103.33		10, bl
+Mn	535.2	100.18		10, br	1,144.0	99.31		9, bl	1,593.8	95.02		9, bl	991.3	98.24		4, bl	1,039.7	103.69		10, bl
+Mo	542.7	101.58		10, br	1,159.2	100.62		9, bl	1,648.9	98.25		9, bl	1,016.6	100.74		4, bl	1,032.9	103.01		10, bl
+N	562.9	105.37		10, br	1,142.3	99.16		4, bl	1,661.6	100.18		9, bl	[2] 1,028.9	101.84		2, bl	[2] 1,035.3	103.24		10, bl
+K, P	520.2	97.44		10, br	1,119.2	97.15		9, bl	1,692.2	102.03		10, bl	938.9	93.03		4, bl	1,119.4	111.63		10, bl
+Mg, S	537.0	100.53		10, br	1,152.1	100.01		9, bl	1,681.3	101.36		10, bl	1,016.9	100.76		4, bl	1,026.3	102.34		10, bl
Max.[3]	562.9				1,203.0				1,692.2				1,028.9				1,119.4			
C. U.[4]		45.03				48.12				45.12				41.16				44.78		
pH[5]			7.13				7.15				7.18				7.13				7.45	

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[1] Sporulation is indicated on a scale of 0 (none) to 10 (complete), and spore color by the initial letter or letters of the words black, brown, tan, and white

[2] Increased acidity with sulphuric acid, without addition of nitrogen.

[3] Maximum individual yield.

[4] Coefficient of utilization or yield per 100 g of sucrose

[5] Initial acidity of the nutrient solution.

TABLE 3.—*Mineral content of Aspergillus niger grown for 4 days at 35° C. in optimum solutions (NH_4NO_3) of different sucrose concentrations*

Constituent	Mineral content of organism with—								
	25 g of sucrose per liter			50 g of sucrose per liter.			75 g of sucrose per liter		
	In mycelial felts dried at 103° C.	Quantity of [1] constituent—		In mycelial felts dried at 103° C.	Quantity of [1] constituent—		In mycelial felts dried at 103° C.	Quantity of [1] constituent—	
		Supplied [2] per liter	Absorbed by organism		Supplied [2] per liter	Absorbed by organism		Supplied [2] per liter	Absorbed by organism
	Percent	*Mg*	*Percent*	*Percent*	*Mg*	*Percent*	*Percent*	*Mg*	*Percent*
Ash	1.55	210.0	78.9	1.65	453.8	83.8	1.88	827.4	75.4
Nitrogen	2.62	308.0	90.9	2.83	665.0	98.0	2.98	1,067.5	92.6
Potassium	.61	76.3	85.1	.59	157.2	86.5	.60	269.4	73.9
Phosphorus	.26	30.3	91.7	.26	62.3	96.1	.31	106.8	96.3
Magnesium	.05	9.9	53.5	.05	24.8	46.4	.07	52.5	44.2
Sulphur	.08	13.0	65.8	.10	32.5	70.9	.13	68.9	62.6

[1] Computed on the basis of the quantities given in tables 1 and 2 for nutrient-solution constituents and yields of oven-dried tissue.

[2] The quantity of ash supplied is a computed value and does not include volatile constituents, such as ammonium nitrate, water of crystallization, and water lost through conversion of orthophosphate to pyrophosphate on ashing. The ash was quite deliquescent and free from carbonate.

NITROGEN GROWTH CURVES

The data showing the influence of the quantity of nitrogen on the growth of the fungus have been tabulated in table 4. These data will be discussed subsequently at greater length with the aid of graphic illustrations. The question of precision in results is best studied at this point, however.

The total number of pairs of duplicates is 120. Table 4 shows the deviation values for 119 pairs. Of these, 54, or 45 percent, deviated from the average by no more than ±1 percent; 81, or 68 percent, by no more than ±2 percent; 97, or 82 percent, by no more than ±3 percent; and 113, or 95 percent, by no more than ±5 percent. The maximum variation was ±9.1 percent. That this precision is subject to improvement is possible, since the procedures used were the ordinary methods, devised for speed as well as accuracy. It is, at any rate, definitely superior to the precision attainable in growth studies with green plants, as well as that formerly considered acceptable for work with *Aspergillus*. Ekman (*2*) studied the precision of his experimental data with this organism and found that of 130 duplicate determinations only 61 percent varied by no more than ±5 percent. Four of his duplicates varied by ±25 percent.

Reproducibility of results in successive experiments is of course the more important criterion of accuracy. The two experiments with varying nitrogen at a sucrose concentration of 7.5 percent afford material for a comparison, if the values in the second experiment are computed for nitrogen concentrations used in the first. They were performed a month apart. For the 19 values corresponding to those of the first determination, the deviations from the average were as follows: 5, or 26 percent of the total, vary by less than ±1 percent; 10, or 53 percent of the total, by less than ±2 percent; and 14, or 74 percent of the total, by less than ±3 percent. In none of the 19 cases did the variation exceed ±5 percent. Acidity values (pH), though quite uniform with the lower nitrogen concentrations, varied by as much as 0.5 of a unit at the higher concentrations. Increased experimental precision should give improvement in this respect also, but may be difficult to attain without a knowledge of all the essential elements.

TABLE 4.—*Yields of Aspergillus niger with graduated quantities of nitrogen when grown for 4 days at 35° C.*

Nitrogen per liter	Individual cultures		Average	Deviation	Acidity	Nitrogen per liter	Individual cultures		Average	Deviation	Acidity
2 5-percent sucrose solution+NH_4NO_3 (pH=7.16)						5.0-percent sucrose solution+NH_4NO_3 (pH=7 32)					
Mg	*Mg*	*Mg*	*Mg*	*Percent*	*pH*	*Mg*	*Mg*	*Mg*	*Mg*	*Percent*	*pH*
0	0 0	0 0	0 0	0 0	4 55	0	0.0	0 0	0 0	0 0	5 37
20	34 1	33 1	33.6	±1 5	2.71	40	66 9	73 6	70 3	±4 7	2 70
40	73 9	66 8	70 4	±5 1	2 66	80	153 0	146 1	149 6	±2 3	2 58
60	113.7	116 6	115 2	±1 2	2 64	120	238 1	235 0	236 6	± 7	2 56
80	153 4	141 8	147 6	±3 9	2 65	160	307 1	309 7	308 4	± 4	2 55
100	210 0	218 7	214 4	±2 0	2 63	200	408.1	399 5	403 8	±1 1	2 55
120	229 8	252 6	241 2	±4 7	2 70	240	466 0	497 2	481.6	±3 2	2 53
140	233 9	277 2	255 6	±8 4	2.64	280	552 4	546 6	549 5	± 5	2 46
160	284 7	290 2	287 5	± 9	2 55	320	605 9	617 4	611 7	± 9	2 39
180	304 7	311 4	308.1	±1 1	2 44	360	661 6	644 3	653 0	±1 3	2 32
200	328 3	358 8	343 6	±4 4	2 28	400	700 0	712 0	706 0	± 9	2 25
220	394 4	417 0	405 7	±2 8	2 38	440	796 9	803 9	800 4	± 4	2 28
240	405 4	440 6	423.0	±4 2	2 44	480	879 5	907 9	893.7	±1.6	2 38
260	493 3	486 2	489 8	± 8	2 58	520	989 6	958.2	973 9	±1 6	2 58
280	504 0	511 5	507.8	± 7	2 68	560	1,012 2	1,046 0	1,029 1	±1 6	2 70
300	536 7	507 4	522 1	±2 8	2 83	600	1,056 0	997 2	1,026 6	±2 9	2 86
320	549 7	542 0	545.9	± 7	3 13	640	1,079 0	1,001 3	1,040.2	±3.7	3 03
340	547 9	562 9	555 4	±1.4	2 57	680	1,099 1	1,109 5	1,104 3	± 5	2 86
360	551 6	562 0	556.8	± 9	2 41	720	1,114 8	1,131 3	1,123 1	± 7	2 75
380	539 4	554 2	546 8	±1.4	2 11	760	1,101 9	1,102 6	1,102 3	.0	2 47
7 5-percent sucrose solution+NH_4NO_3 (pH=7 38)						7.5-percent sucrose solution+NH_4NO_3 (pH=7 28)					
0	0 0	0 0	0 0	0 0	6 33	80	135 1	162 2	148 7	±9 1	2 65
60	115 9	113 0	114 5	±1 3	2 72	160	296 7	299 6	298 4	± 4	2 54
120	243 4	240.0	241 7	± 7	2 61	240	483 0	473 8	478 4	±1 0	2 50
180	370 8	386 2	378 5	±2 0	2 64	320	646 1	641 9	644 0	± 3	2 45
240	465 7	489 5	477 6	±2 5	2 54	400	764 7	755 7	760 2	± 6	2 40
300	581 6	622.0	601 8	±3 4	2 48	480	938 6	913 4	926 0	±1 4	2.35
360	716 4	747 6	732 0	±2 1	2 44	560	1,063 1	(1,211 0)	1,063 1	(±6 5)	2 39
420	754 2	834 0	794 1	±5 0	2 37	640	1,192 1	1,200 7	1,196 4	± 4	2 39
480	844 3	873.9	859 1	±1 7	2 33	720	1,333 6	1,269 0	1,301 3	±2 5	2.35
540	912 5	973 5	943.0	±3 2	2 28	800	1,444 7	1,376 6	1,410 7	±2 4	2 33
600	1,023 3	1,064 2	1,043 8	±2 0	2.24	880	1,474 8	1,500 6	1,487 7	± 9	2 29
660	1,180 7	1,208.4	1,194 6	±1 2	2 28	960	1,542 2	1,524 8	1,533 5	± 6	2 69
720	1,273 6	1,356 0	1,314.8	±3 1	2.34	1,040	1,602 5	1,574 4	1,588 5	± 9	2 95
780	1,442 2	1,438 8	1,440 5	± 1	2 55	1,120	1,612 8	1,613 2	1,613 0	0	3 25
840	1,518 2	1,494.3	1,506 3	± 8	2 68	1,200	1,597 4	1,554 6	1,576 0	±1 4	3 24
900	1,531 9	(1,221 4)	1,531 9	?	3 03	1,280	1,565 8	1,546 1	1,556 0	±.6	2 71
960	1,550 5	1,503 9	1,527 2	±1 5	3 16	1,360	1,537 4	1,535 4	1,536.4	± 1	2 42
1,020	1,583 5	1,546.4	1,565 0	±1 2	3 32	1,440	1,580 2	1,506 9	1,543 6	±2 4	2 71
1,080	1,593 9	1,562.3	1,578 1	±1 0	3 47	1,520	1,550 1	1,547 1	1,548 6	±.1	2.44
1,140	1,604 3	1,586.3	1,595 3	± 6	2 99	1,600	1,576.6	1,587 1	1,581 9	± 3	2 08
5 0-percent sucrose solution+NH_4Cl (pH=7 44)						5 0-percent sucrose solution+$NaNO_3$ (pH=7 44)					
38	67 8	67 9	67.9	±0.1	2 74	34	53 0	55 7	54 4	±2 6	2 82
76	171.3	150 9	161 1	±6.3	2.46	68	123 7	122 7	123.2	± 4	2 72
114	226.7	231 7	229 2	±1.1	2 27	112	197 8	185.8	191 8	± 3	2 71
152	276 5	302 3	289 4	±4.5	2 14	146	247 2	234 8	241 0	±2.6	2 71
190	357.5	345 1	351 3	±1 8	2.03	180	310 8	318 6	314.7	±1.2	2 76
228	416.5	442.4	429.5	±3.0	1.93	214	372 8	367 4	370 1	± 7	2.76
266	466.0	473.2	469.6	± 8	1 88	248	379 7	399 7	389.7	±2 6	2 81
304	534 6	539.8	537.2	± 5	1.81	282	442 6	463.0	452 8	±2 3	2.83
342	595.6	596.0	595 8	.0	1.75	316	473.9	477.0	475 5	± 3	2 86
380	672 7	657 6	665 2	±1.1	1.68	350	542.6	551 3	547.0	± 8	2.84
418	706.0	784.9	745 5	±5.3	1 61	384	584 6	561.3	573.0	±2.1	2 62
456	913.4	851.0	882.2	±3.5	1.60	418	628.8	596.8	612 8	±.3	2 36
494	866 3	857.4	861.9	±.5	1.55	452	636.8	679.6	658 2	±3 3	2 03
532	901.4	974.9	938 2	±3.9	1.53	486	701.8	685 1	693 5	±1 4	2 21
570	845 2	854 5	849.9	±.6	1 53	520	774 3	775 7	775 0	± 1	2 08
608	990 8	978.4	984 6	±.6	1.48	554	793.6	737 0	765.3	±3 7	2 03
646	1,028.5	991 1	1,009.8	±1.9	1 41	588	823 8	824.9	824.4	±.1	2 08
684	1,010.7	1,019.0	1,014.9	±.4	1.42	622	857.4	846.8	852.1	±.6	2 05
722	1,050.7	1,004.1	1,027.4	±2.3	1.42	656	895.5	885 9	890 7	±.5	2.05
760	1,074.0	1,064 1	1,069.1	±.5	1.39	690	942 3	920.3	931 3	±1.2	2.08

NITROGEN PARTITION IN THE CULTURES

The nitrogen content (total) of the mycelial felts grown with ammonium nitrate at the three levels of carbohydrate nutrition has been summarized in table 5. The percentage of nitrogen in the dried tissues of the organism is fairly uniform for analogous concentrations. It is slightly lower at the lower nitrogen levels and increases very gradually until the optima for growth are reached. Whether the slight increases in the percentage of nitrogen at the optima when sugar is increased be of significance is problematical. Comparisons between the nitrogen utilized by the organism at identical levels would indicate that nitrogen was the limiting factor for growth, since the amount of nitrogen absorbed was influenced but little by the concentration of the other constituents present. For example, the amount of nitrogen removed from the solution at the 360-mg level with 2.5 percent of sucrose was 15.4 mg; with 5.0 percent of sucrose, 16.0 mg; and with 7.5 percent of sucrose, 14.0 mg.

TABLE 5.—*Distribution of nitrogen in cultures of Aspergillus niger with graduated quantities of nitrogen when grown for 4 days at 35° C.*

2.5 percent of sucrose					5.0 percent of sucrose					7.5 percent of sucrose				
Nitrogen per liter	Nitrogen in mycelium			Nitrogen used	Nitrogen per liter	Nitrogen in mycelium			Nitrogen used	Nitrogen per liter	Nitrogen in mycelium			Nitrogen used
	Found	Calculated	Per culture			Found	Calculated	Per culture			Found	Calculated	Per culture	
Mg	*Percent*	*Percent*	*Mg*	*Percent*	*Mg*	*Percent*	*Percent*	*Mg*	*Percent*	*Mg*	*Percent*	*Percent*	*Mg*	*Percent*
20	2.169	2.04	0.7	73.0	40	2.063	2.03	1.5	72.5	60	1.923	2.03	2.2	73.4
40	1.981	2.06	1.4	69.5	80	2.030	2.05	3.0	75.9	120	2.035	2.04	4.9	81.7
60	2.033	2.07	2.3	78.0	120	2.072	2.07	4.9	81.7	180	2.035	2.05	7.7	87.8
80	2.055	2.13	3.0	75.8	160	2.109	2.10	6.5	81.3	240	1.742	2.09	8.3	69.3
100	1.819	2.08	3.9	78.0	200	2.100	2.10	8.5	84.8	300	1.622	2.11	9.8	65.1
120	2.105	2.13	5.1	84.7	240	2.190	2.13	10.6	87.8	360	1.834	2.13	13.4	74.6
140	2.350	2.20	6.0	86.0	280	2.277	2.16	12.4	88.7	420	1.988	2.19	15.6	74.3
160	2.392	2.24	6.9	86.0	320	2.338	2.21	14.3	89.4	480	2.100	2.26	18.0	75.2
180	2.429	2.31	7.5	83.1	360	2.448	2.25	16.0	88.8	540	2.157	2.31	20.3	75.3
200	2.521	2.34	8.7	86.6	400	2.532	2.31	17.9	89.4	600	2.297	2.34	24.0	79.9
220	2.348	2.31	9.5	86.6	440	2.213	2.31	17.7	80.5	660	2.353	2.34	28.1	85.2
240	2.456	2.38	10.4	86.6	480	2.213	2.33	19.8	82.4	720	2.381	2.36	31.1	86.9
260	2.296	2.35	11.3	86.5	520	2.269	2.30	22.1	85.0	780	2.283	2.38	32.9	84.3
280	2.383	2.41	12.1	86.4	560	2.367	2.40	24.4	87.0	840	2.381	2.45	35.9	85.4
300	2.538	2.49	13.3	88.3	600	2.494	2.51	25.6	85.3	900	2.760	2.54	42.3	94.0
320	2.482	2.55	13.6	84.7	640	2.634	2.60	27.4	85.6	960	2.718	2.66	41.5	86.4
340	2.632	2.64	14.6	86.0	680	2.620	2.64	28.9	85.1	1,020	2.971	2.74	46.5	91.2
360	2.757	2.74	15.4	85.3	720	2.760	2.72	31.0	86.1	1,080	3.097	2.84	48.9	90.5
380	2.842	2.87	15.5	81.8	760	2.971	2.80	32.8	86.2	1,140	3.069	2.94	49.0	85.9
7.5 percent of sucrose					7.5 percent of sucrose (adjusted values)					7.5 percent of sucrose (average)				
80	2.019	2.04	3.0	75.1	60	2.005		2.4	74.4	60	1.964		2.3	73.9
160	2.076	2.08	6.2	77.8	120	2.048		4.6	76.5	120	2.042		4.8	79.1
240	2.112	2.09	10.1	84.2	180	2.085		7.2	79.4	180	2.060		7.4	83.6
320	2.075	2.12	13.4	83.5	240	2.112		10.1	84.2	240	1.927		9.2	76.8
400	2.058	2.18	15.6	78.2	300	2.084		12.6	83.6	300	1.853		11.2	74.4
480	2.058	2.20	19.1	77.6	360	2.067		14.5	80.9	360	1.951		14.0	77.8
560	2.086	2.25	22.2	75.6	420	2.058		16.5	78.0	420	2.023		16.1	76.2
640	2.255	2.30	27.0	84.3	480	2.058		19.1	77.6	480	2.079		18.6	76.4
720	2.297	2.34	29.9	83.0	540	2.079		21.4	76.1	540	2.118		20.9	75.7
800	2.381	2.44	33.6	84.0	600	2.171		24.6	80.0	600	2.234		24.3	80.0
880	2.494	2.52	37.1	84.3	660	2.266		27.7	84.0	660	2.310		27.9	84.6
960	2.662	2.64	40.8	85.0	720	2.297		29.9	83.0	720	2.339		30.6	85.0
1,040	2.746	2.76	43.6	83.9	780	2.360		32.7	83.8	780	2.325		32.8	84.1
1,120	3.041	2.88	49.1	87.6	840	2.438		35.4	84.2	840	2.410		35.6	84.8
1,200	3.294	3.07	51.9	86.5	900	2.536		38.0	84.5	900	2.648		40.2	89.3
1,280	3.490	3.23	54.3	84.9	960	2.662		40.8	85.0	960	2.690		41.2	85.7
1,360	3.462	3.38	53.2	78.2	1,020	2.725		42.9	84.1	1,020	2.848		44.7	87.7
1,440	3.673	3.53	56.7	78.7	1,080	2.894		46.3	85.8	1,080	2.996		47.6	88.2
1,520	3.771	3.67	58.4	76.8	1,140	3.103		49.8	87.3	1,140	3.086		49.4	86.6
1,600	3.869	3.81	61.2	76.5										

Computation of the percentages of nitrogen not absorbed gave values of much interest that were not anticipated. Intake seemed to be at a minimum with minimum nitrogen supply. Even at concentrations about 5 percent of optimum only about 75 percent of the nitrogen was utilized. Percentages of nitrogen absorbed are in accordance, nevertheless, with the observation of Roberg (*10*) that this fungus cannot remove iron completely from solution even at extreme deficiency levels.

The data of table 5 also afford a basis for an interesting analysis with respect to total nitrogen. If the assumption is made that protoplasmic nitrogen in the mycelial felts is fairly constant, the excess nitrogen in the mycelial felts must be absorbed nitrogen that has not become an integral part of the protoplasm. The fungus mycelium is, of course, in close contact with its nutrient solution and probably does not store nitrogen in any form. The partition of the nonprotoplasmic nitrogen in the culture, if similar to that of a compound between two immiscible solvents, would be proportional to the coefficient of distribution and the relative quantities of felt and solution in the protoplast-nutrient solution system. Calculations for total nitrogen in the dry mycelial felts were made on this basis for each chemical determination of total nitrogen. Nonprotoplasmic nitrogen was taken to equal the difference between the amount added and the 2-percent content of the mycelia under starvation conditions. Of this quantity, $0.6 \frac{\text{yield}}{\text{maximum yield}} \times$ nonprotoplasmic nitrogen equals the nitrogen present passively in the organism. The constant equaling 0.6 corresponds to a coefficient of distribution of 1.5, and implies that the ammonium nitrate was one and one-half times more soluble in the protoplast than in the solution. The computed percentages of total nitrogen in the mycelial felts agree with the determined values within the limits of experimental and chemical precision. The values for found and calculated percentages of total nitrogen did not differ by more than approximately 0.1 percent of total nitrogen in 48 out of 77 determinations (62 percent). Sixty-six, or 86 percent, agreed within 0.2 percent of total nitrogen. Moreover, the 11 remaining values were just those representing determinations that seemed irregular. If not a proof of the correctness of the above premises, it must, nevertheless, be granted that they afford an empirical means for the accurate computation of total nitrogen under these experimental conditions.

RELATIVE YIELD AND NITROGEN CONTENT AT DIFFERENT SUCROSE LEVELS

Superimposition of the corresponding curves representing yield and nitrogen absorbed for the three levels of carbohydrate nutrition was brought about in figure 1 by multiplying all values by 2 at the 2.5-percent level, by 1 at the 5.0-percent level, and by two-thirds at the 7.5-percent level. There would seem to be little doubt that a high degree of coincidence exists, especially if due allowance be made for probable errors in the determination of the values. The yield curves are similar in form to those frequently reported but show definite deviations from uniformity. All four curves with ammonium nitrate are slightly concave to the X-axis at minimum concentrations, and slightly convex at moderate concentrations. The graphs for nitrogen utilization seem to be linear.

Figure 2 gives a comparison of the yields obtained in a 5.0-percent sucrose solution with ammonium nitrogen, with nitrate nitrogen, and with equal amounts of each (NH_4NO_3). Though maximum yields are the same with all these sources of nitrogen (*13*), the intermediate values differ appreciably. Ammonium nitrate gave the highest intermediate yields, sodium nitrate the lowest intermediate yields, while ammonium chloride gave median yields. These differences in yield would seem to be correlated with the acidities of the nutrient solution, especially if it is assumed that the use of ammonium chloride results in excessive acidities at intermediate nitrogen-deficiency levels.

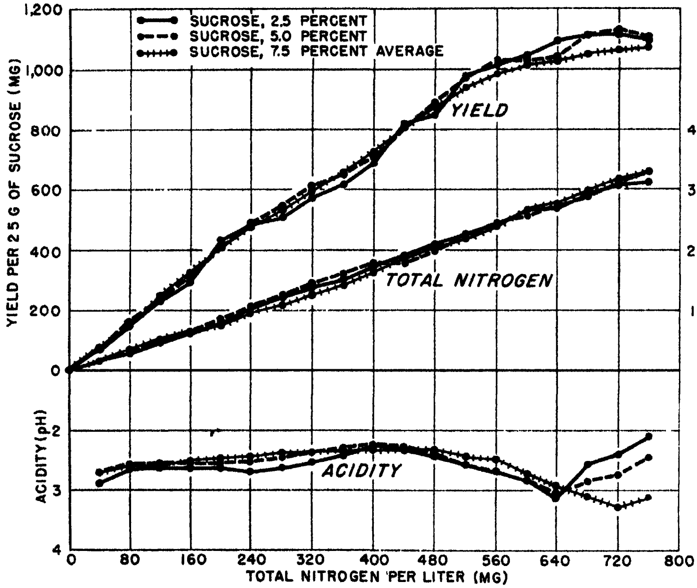

Figure 1.—Superimposed curves for yield, total nitrogen content, and acidities at harvest when graduated quantities of nitrogen as ammonium nitrate were used with *Aspergillus niger* grown for 4 days at 35° C. Superimposition was brought about by multiplying all values, except acidity, by 2 for 2.5 percent of sucrose, by 1 for 5.0 percent of sucrose, and by two-thirds for 7.5 percent of sucrose.

The acidities of the culture solutions at harvest are of course the resultant of residual electrolytes and organic acids synthesized by the fungus. The favorable effects of ammonium nitrate, if attributable to acidity, might follow through the selective utilization of the ammonium and nitrate ions.

THEORETICAL GROWTH CURVE FOR NITROGEN

Without doubt the growth formula suggested by Mitscherlich (*11*) has aroused most discussion and led to more work than any other. Mitscherlich claimed that the constants of his equation have a definite biological meaning and that the value of c in the formula $y=A(1-10^{-cx})$ is dependent only on the type of fertilizer or growth factor. The value A represents the maximum yield, and y the yield with a quantity (x) of nitrogen, phosphorus, etc. Mitscherlich (*11*), despite his claims for the theoretical accuracy of his formula, later found it necessary to modify the above expression to $y=A(1-10^{-cx})\ 10^{-kx^2}$ in order to make his formula suitable for computing negative increments in yield with excess fertilizer. Extensive data have been

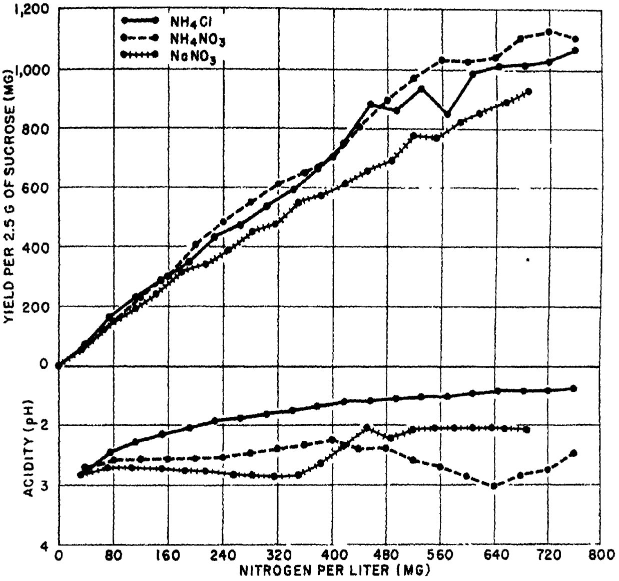

Figure 2.—Yield and acidity curves when graduated quantities of nitrogen, supplied as ammonium chloride, ammonium nitrate, and sodium nitrate were used with *Aspergillus niger* grown for 4 days at 35° C. The sucrose level was 5 percent.

accumulated by Meyer (*6*) with *Aspergillus niger* for use in the mathematical analysis of the Mitscherlich formula.

A brief study of the data herein presented has been made in this connection and is represented by the graphs of figure 3. Curves *a* and *b* are replicate yield curves for ammonium nitrate and 7.5 percent of sucrose, obtained a month apart. Their values are averaged in curve *c*. The original Mitscherlich equation is represented by curve *d*, the value for the maximum yield (A=4,837.6) being computed for nitrogen by his method. Curves *e* and *f* are also Mitscherlich yield graphs computed for the maximum yield (1,600) obtained experimentally. The remaining curve (*g*) represents an empirical equation sometimes used to express engineering data.

It is evident that none of the computed curves agree with the experimental growth curve. The Mitscherlich equation of growth can be made to agree quite closely with the lower yield values (*d*) or the maximum value (*e, f*), but it cannot be made to give figures in agreement with all values of the experimental curve. Use of the modified Mitscherlich formula gave results but little better than those of the empirical curve (*g*) for equation $y=a-b\ (c-x)^n$, in which y represents yield, x the nitrogen supply, and a, b, c, and n are constants.

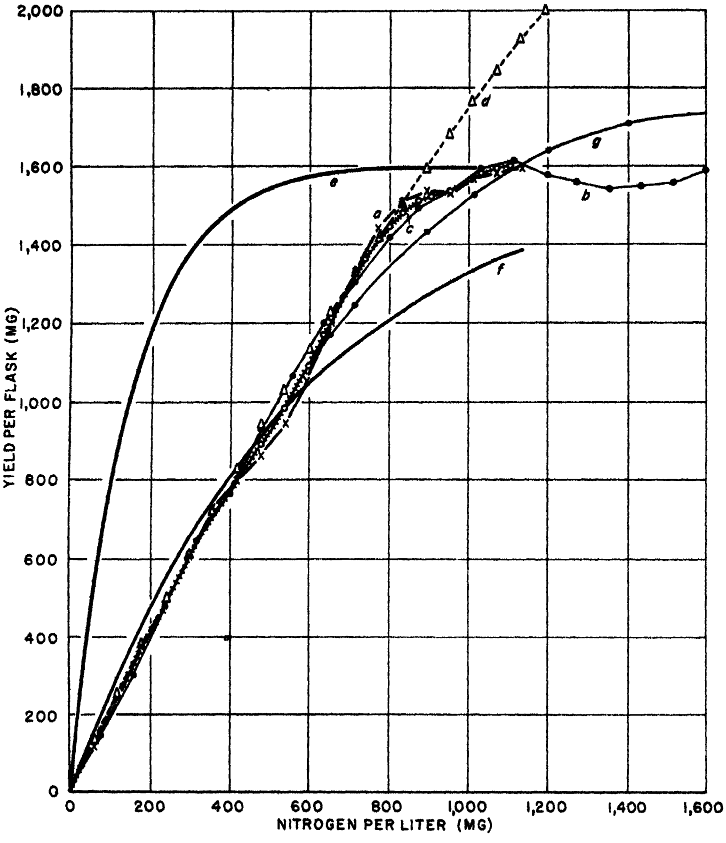

FIGURE 3.—Computed and experimental yields when graduated quantities of nitrogen were used with *Aspergillus niger* grown for 4 days at 35° C. in an optimum solution containing 7.5 percent of sucrose. Curves *a* and *b* are replicate determinations, and curve *c* is their average. Curves *d*, *e*, and *f* were computed with the Mitscherlich growth formula $y=A\ (1-10^{-cx})$. In curve *d*, A equals 4,837.6 and c is 0.0001927. The constants for curve *e* are $A=1{,}600$ and $c=0.0028$; and for curve *f*, $A=1{,}600$ and $c=0.000761$. The constants for the empirical equation of curve *g* are, in formula form, $y-1{,}725=-0.0001493\ (1{,}632.5-x)^{2.2}$.

DISCUSSION

Variation of nitrogen supply in optimum solutions at the three levels of sugar nutrition led to what are essentially identical results.

Graduated quantities of this element ranging from 0 to optimum gave in all cases yield curves of exponentiallike form and total-nitrogen curves of linear form that could be mutually superimposed quite closely. The yields and the quantities of nitrogen absorbed at all deficiency levels were nearly exactly proportional to the quantity supplied irrespective of the other constituents. In other words, the variations relative to the respective maxima were identical, and the maxima therefore are satisfactory for use as fixed physiological standards of reference. Later replication of these standard solutions, moreover, is readily possible with a satisfactory degree of accuracy, if the relative salt concentrations (1 : 2 : 3) required by the three optimum solutions with ammonium nitrate and relative yields furnish a criterion.

It was found possible to compute total nitrogen in the felts at nitrogen levels up to optimum, with a precision equal to that of the chemical determinations. The assumptions on which these calculations are based afford, therefore, a possible explanation of certain phases of nitrogen absorption and content of this organism. That is to say, the total nitrogen in *Aspergillus niger* is probably composed of a relatively fixed percentage of protoplasmic nitrogen and a variable proportion of absorbed nutritive nitrogen. The quantity of the latter component in the felts is dependent on the concentration of nonprotoplasmic nitrogen available. Its distribution between organism and solution seems to be entirely in accordance with the physicochemical laws governing distribution of dissolved substances between immiscible solvents.

It is doubtful, however, that the results on absorption could hold for green plants in view of the morphological differences that exist. Their lack of direct and practically complete contact with the nutrient solution and their capacity for nitrogen storage have indeed led to results of a different character (*5*, *7*). Nevertheless, these results render it possible that biological absorption in its simplest form may be entirely passive, even for negative protoplast-solution gradients, and based on the relative solubilities of a substance in the protoplast and solution. The use of a nutrient in concentrations higher than optimum may be expected to lead to changes in the protoplasm that will materially alter these responses. Other additions may be equally effective in this respect. Recognition of these factors does not vitiate the importance of the type of absorption due wholly to solubility, but offers a point of departure in the study of the influence of these factors on absorption.

Though the interpretation of these results on the basis of absorption has been emphasized because of its importance, if correct, to an understanding of these phenomena, it is by no means the only possible explanation. No data are available on the form and location of the nitrogen in the tissues. The appearance, quantity, and composition of the cell-wall material of the hyphae undergo a regular change within the 0-to-optimum nitrogen range. Since nitrogen enters into the composition of the cell wall of this fungus, the importance of obtaining additional information before coming to a final decision can be realized. Despite the fact, however, that either or both of these views may be right, the regularity of the changes in composition and their ease of duplication would warrant additional investigation.

The yield curves with ammonium and nitrate nitrogen afford a concrete illustration of frequent error in quantitative comparisons of utility with nutrients. The ratios in yield with these two sources of nitrogen vary throughout the whole range, even though the optimum nitrogen concentrations and the maximum yields for both are identical (*13*). That even more striking differences may occur in the absence of proper concentrations of the trace elements has been demonstrated in the paper just cited. Prior knowledge of the physiological effect of each constituent and of its concentration is a necessary requirement, therefore, for the interpretation of studies in nutrient efficiency.

The quantitative concordance in relative results for yields, acidity, nitrogen absorbed, and the percentage of nitrogen used in the three optimum solutions when the nitrogen supply was varied is considered to be a demonstration of the suitability of results with an optimum solution of minimum salt content as biological reference standard. The constancy in mineral contents of the fungus and in the percentages at which they were utilized furnish additional evidence to strengthen this interpretation. Ordinarily a variation in supply of one mineral constituent results in a variation in the percentages of the others found in the tissues. Many instances of this type of behavior have been determined by Rennerfelt (*9*) at a concentration range of 1 to 100. Employment by this investigator of an optimum solution of minimum salt content would, it is believed, have led to different results in certain instances. With the three optimum solutions having sucrose contents as 1, 2, 3, the mineral requirements and the yields bore the same proportions. The mineral composition of the fungus felts grown on these solutions and the percentages of mineral utilization were identical, within experimental precision, in all three instances.

These results also lead to the impression that the formula advocated by Mitscherlich (*11*), though showing close agreement with certain portions of the nitrogen growth curve of *Aspergillus niger*, has no general theoretical significance. Even the new formula is but slightly better for representing yields than a purely empirical equation selected almost at random. Furthermore the growth curve for nitrogen (NH_4NO_3) is not uniform but has several points of inflection that do not lend themselves to mathematical expression with the comparatively simple formulations so far proposed. The computed value of *A*, the maximum yield, is, for nitrogen at least, of no biological significance with *Aspergillus*. The view of Mitscherlich (*11*) that the maximum yield for nitrogen can never be obtained in practice but must be computed fails to be convincing when it necessitates assuming that *A. niger*, with a total quantity of 3,750 mg of sucrose per culture, should be capable of giving a maximum yield of 4,838 mg. Actually, of course, the situation is that the Mitscherlich curve may be computed to agree with many of the lower values sufficiently for practical purposes but cannot be computed to agree with all the experimental yields in one and the same curve. A similar situation was found to exist by Garner et al. (*3*) with tobacco. In substance, rejection of the experimentally determined nitrogen maximum amounts to rejection of the experimental criteria for accuracy of the calculated yields in order to eliminate a disagreement in results.

There can be little doubt that the points of inflection found in the nitrogen growth curve for *Aspergillus* have an actual existence. They occur in every experimental curve with ammonium nitrate as the source of nitrogen, even though the sugar concentration varied as 1 to 3, and may be associated with changes in the relative rates of absorption of ammonium and nitrate ion. Yields with ammonium chloride and with sodium nitrate do not show similar divergencies. The relative positions of these growth curves for *Aspergillus* are the same as that found by Garner et al. (*3*) for the tobacco plant.

A comprehensive analysis of the agreement between found and computed yields at all deficiency levels cannot be made, however. Though the variation between duplicate yields and between replicate determinations is only ±5 percent or less, this accuracy is insufficient for the proper evaluation of the slight differences that exist for the lower values. At least double the precision is necessary, i. e., a variation of less than ±2 percent in replicate trials. Experimental determinations within this margin of error should be obtainable when all the essential elements have been identified and procedures are based solely on precision in results.

SUMMARY

Yield curves with graduated quantities of nitrogen were obtained with *Aspergillus niger* grown in optimum nutrient solutions for 4 days at 35° C. Forty-five percent of the duplicate cultures agreed to within ±1 percent, 68 percent to within ±2 percent, 82 percent to within ±3 percent, and 95 percent to within ±5 percent. Yields could be verified to within ±5 percent on repetition. Growth with ammonium nitrate was studied at three levels of carbohydrate nutrition and was compared with growth obtained with ammonium chloride and with sodium nitrate. The growth and total-nitrogen curves obtained with 2.5, 5.0, and 7.5 percent of sucrose were found to be proportional and identical in form when superimposed. Other results for the three solutions were also practically identical. At intermediate nitrogen concentrations, ammonium chloride gave lower and sodium nitrate still lower yields than did ammonium nitrate. Total nitrogen in the mycelial felts grown with ammonium nitrate increased linearly with increasing concentrations of nitrogen. Values for total nitrogen in the felts could be computed within the limits of accuracy for the chemical determinations. The assumption was made that the fungus invariably contained 2 percent of protoplasmic nitrogen and that the balance of nitrogen supplied was partitioned between the organism and the solution similarly to the distribution of a dissolved substance between two immiscible solvents. The values of yield curves with ammonium nitrate could not be duplicated by computation with the Mitscherlich equation of growth.

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INFLUENCE OF VARIOUS FACTORS ON THE SHAPE OF BEET ROOTS[1]

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INTRODUCTION

To one acquainted with varieties of table beets (*Beta vulgaris* L.) no close examination is necessary to observe that individual roots within a variety represent many distinct types of root shape. On account of the difficulties involved in producing inbred beet seed, very few commercial strains are highly uniform for any morphological character. The great variation in shape of roots within a single strain raises the question as to its cause; doubtless in many cases it is due to genetic factors. The present studies were undertaken to determine whether certain environmental factors, including soil texture, soil fertility, and seasonal conditions correlated with time of planting, induce variations in the shape of beet roots that can be determined by shape indices.

Numerous investigations have been reported in which the shapes of various plant organs have been described in terms of numerical indices, including studies of the variation in shape of roots in some of the root crops. Edmond (*3*)[3] found that the depth to which the seeds were covered in planting had a measurable effect on the shape of radish roots. Mack and Lachman (*6*) presented data to show that consistent root-shape differences between beet varieties can be shown by numerical indices. Schermerhorn (*7*), Zimmerley (*9*), Carolus (*2*), and Boswell and Beattie (*1*) reported on the influence of different fertilizer treatments on the shape of sweetpotato roots as determined by an index obtained by comparing the polar and equatorial diameters. Pino[4] found that the shape of beet roots was influenced by the proportion that developed above and below the soil surface, and Hummel (*5*) worked out a comprehensive system of recording and interpolating root shapes.

PROCEDURE

PLANTINGS

Studies on root shape were confined to one strain of the beet variety Detroit Dark Red. In order to eliminate as far as possible variation due to genetic factors, a strain[5] was selected that was

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[2] The writer wishes to acknowledge his indebtedness to V R Boswell, of the Division of Fruit and Vegetable Crops and Diseases, for suggesting the problem and for helpful criticism throughout the investigations, and to Lester E. Barrett and William F. Kosar, of the same Division, for valuable assistance in recording the data and computing the indices

[3] Italic numbers in parentheses refer to Literature Cited, p 745

[4] Pino, A. L. A STUDY OF THE TABLE BEET IN AN ATTEMPT TO DISCOVER THE CONDITIONS, CLIMATIC AND CULTURAL, WHICH CAUSE ITS DISCOLORATION. Unpublished thesis, Mich. State Col Agr. and Appl. Sci. East Lansing, Mich. 1928.

[5] One of the most uniform strains in root shape in the vegetable standardization trials of the Division of Fruit and Vegetable Crops and Diseases.

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known to produce roots of fairly uniform shape. The same strain was used throughout the investigations.

Root samples for these studies were harvested from 17 crops of beets grown over a period of 5 years; 6 of these crops were grown at Arlington Experiment Farm, Arlington, Va., and 11 at the United States Horticultural Station, Beltsville, Md. (table 1).

TABLE 1.—*Field record with planting and harvesting dates for the 17 crops of beets studied*

Location	Soil type	Moisture	Date planted	Date harvested
Arlington, Va.	Medium loam	Overhead irrigation	Mar. 19, 1933	June 8, 1933
			Apr. 27, 1933	Aug. 11, 1933
			Apr. 9, 1934	July 3, 1934
			May 9, 1934	Aug 14, 1934
			June 7, 1934	Aug. 24, 1934
			Aug. 1, 1934	Oct. 12, 1934
Beltsville, Md.	Deep sand to clay loam	Natural precipitation	May 2, 1933	Aug. 2, 1933
			Apr. 12, 1934	June 26, 1934
			June 6, 1934	Aug. 7, 1934
Do.	Medium loam	Overhead irrigation	May 8, 1935	July 2, 1935
			June 4, 1935	Aug. 6, 1935
			July 9, 1935	Sept. 15, 1935
			Aug. 5, 1935	Oct. 11, 1935
			June 19, 1937	Aug. 23, 1937
			July 19, 1937	Sept 28, 1937
			July 30, 1937	Sept. 30, 1937
			Aug. 23, 1937	Nov. 5, 1937

The six crops grown at Arlington Farm were on a medium loam soil of good fertility, ranging in pH value from 5.6 to 6.2. In both years the land was spring-plowed and fertilized at the rate of 1,500 pounds of 5–8–5 fertilizer per acre. The plots were provided with overhead irrigation and none was permitted to suffer for want of moisture.

The three crops grown at Beltsville in 1933 and 1934 were on land not equipped for irrigation and some were grown with a very limited moisture supply. The field used at Beltsville in 1933 and 1934 was long and narrow, having a soil gradient ranging from deep, loose sand at one end to heavy, reddish clay at the other. This area was comparatively low in fertility previous to the treatments given the fertilizer plots. Three treatments were used and each was duplicated. The plots receiving treatment 1 were given an application of well-decomposed stable manure at the rate of 10 tons per acre, supplemented with a 5–8–5 commercial fertilizer at the rate of 500 pounds per acre. The plots in treatment 2 received the 5–8–5 fertilizer at the rate of 1 ton per acre. In treatment 3 the plots were given an application of 500 pounds of 5–8–5 fertilizer. The plots were arranged in the field so that light sandy soil and heavy clay soil were included in each fertilizer treatment. The reaction of this soil ranged from pH 5.8 to 6.3.

The eight crops grown at Beltsville in 1935 and 1937 were produced on a medium loam soil of high fertility, having a pH range of 5.6 to 6.3. The soil was spring-plowed and fertilized with 5–8–5 fertilizer at the rate of 1,500 pounds per acre. This land was provided with overhead irrigation.

All of the 17 crops were grown in rows 18 inches apart. The planting was done with a mechanical seeder set to cover the seed to a depth of approximately three-quarters of an inch. When the seedlings had reached a height of about 2 inches they were thinned to a distance of 2 to 3 inches between plants in the row.

SAMPLING

In order to determine the number of roots required for a representative sample of a population, a study was made of the variability of the mean index values of samples consisting of 50, 100, and 200 roots. Root samples from three different populations were studied. The mean indices for 50-root samples were quite variable; those for the 100-root samples were much less so, and the differences in most cases were not significant. Very little reduction in variability in mean indices was obtained by increasing the sample from 100 to 200 roots. A random sample of 100 roots was therefore considered representative of a population.

The first data recorded were taken on samples of 100 roots, harvested at random, from the various populations to be studied. No attention was paid to the variation in size of the beets within the sample. When these data were analyzed it was found that the size of the root as indicated by its greatest equatorial width was a factor in determining the various shape indices. A large number of measurements were made to determine the influence of size on shape indices, the data for which will be presented later. It was necessary then to alter the method of taking samples so as to eliminate the variation due to size. All samples compared were of the same equatorial diameter.

RECORDING OF SHAPE DATA

After the samples had been harvested, washed, and dried, the top part was removed by a transverse cut at the point of the oldest leaf scar. A part of the lower end was removed by a transverse cut at a point about 2 cm. below the thickened portion. The roots were then halved longitudinally through a plane at right angles to the lateral rootlets. The outline of the cut surface was found to vary greatly, depending on the plane through which the cut was made. In order to obtain comparable data the longitudinal cut was made as nearly as possible through the same plane in relation to the lateral rootlets.

A permanent record of the shape of the individual roots was made by placing the cut surface of each root on a piece of white paper and carefully tracing the outline. This rapid method made it possible to record the shape of a much larger number of roots than could have been handled if all of the measurements had been made from the roots. From these records the necessary measurements were made.

MEASUREMENTS

The following measurements were taken for each root studied:

(1) The equatorial diameter (*d*) was taken at the point of greatest width as indicated by the line *bc* (fig. 1, *A*).

(2) The thickness (*ld*) of the root was taken at the point of greatest curvature at the base of the thickened portion.

(3) The polar diameter (*a*) was taken as the distance between the lines *ls* and *ld*, at the top and bottom, respectively, of the thickened portion of the root. The line *ls* was determined by the cut made in removing the top at the point of the oldest leaf scar.

(4) Measurements were made of the two portions of the polar diameter (*a*) resulting from its intersection with the equatorial diameter (*d*). The portion above the line *bc* was indicated by the letter *u* and the portion below the line by the letter *m* (fig. 1, *A*).

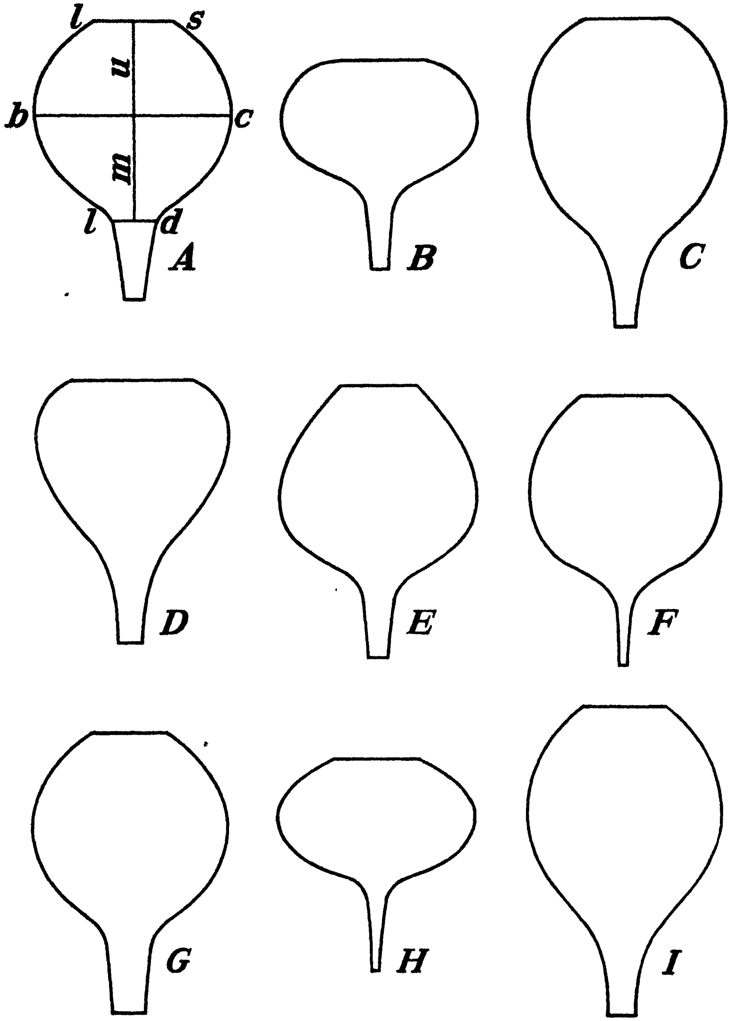

FIGURE 1.—FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE.

Botanically, the underground portion of a beet is largely a thickened taproot. In order to distinguish the thickened upper portion of the beet root from the lower, less thickened portion, the term "taproot" will be used to designate only the lower, unthickened portion below the line *ld*, determined by the point of greatest curvature.

INDICES

From the measurements described in the preceding section, three indices of shape were determined, namely, *a*/*d*, *u*/*m*, and *ld*/*d*.

An index of length (*a*/*d*) was derived by dividing the polar diameter (*a*) by the equatorial diameter (*d*). A value less than 1.00 for *a*/*d* indicates a flattened root; a root having an *a*/*d* index of 0.650 is shown in figure 1, *B*. A high *a*/*d* index indicates a long root; a root having an index of 1.200 is shown in figure 1, *C*. As the *a*/*d* index approaches unity, the root is more nearly spherical (fig. 1, *A*).

A ratio between the polar and equatorial diameters is probably the best single index of shape; however, great variations in shape may occur in beet roots having the same *a*/*d* index, some of which are shown in the outline drawings *D*, *E*, *F*, and *G* of figure 1. The variation in shape in *D* and *E* is due to the position of the intersection of the equatorial diameter (*d*) with the polar diameter (*a*). In order to study this shape variation, an index was derived by dividing the upper portion of the polar diameter (*u*) by the lower portion (*m*). A value less than 1.00 for the *u*/*m* index indicates a top-shaped root in which the greatest bulge is high on the polar diameter. A root of this type, having a *u*/*m* index of 0.400, is shown in figure 1, *D* A value greater than 1.00 for *u*/*m* indicates a root having its greatest bulge low on the polar diameter; that is, with an inverted top-shaped appearance. A root having a *u*/*m* value of 1.500 is shown in figure 1, *E*. As the *u*/*m* index approaches unity, the root is nearly spherical (fig. 1, *A*).

Another shape variation that may occur in beet roots having the same *a*/*d* index is shown in figure 1, *A*, *F*, and *G*. This results from differences in root thickness at the base of the thickened portion of the beet. An index of shape for measuring this type of variation was derived by dividing the root diameter (*ld*) by the equatorial diameter of the thickened portion of the beet (*d*). A small value for the *ld*/*d* index indicates a beet with a small taproot. Such a beet appears more nearly spherical than one having the same *a*/*d* and *u*/*m* indices

EXPLANATORY LEGEND FOR FIGURE 1

Shape indices of beet roots. Factors: Equatorial diameter (*d*), measured by line *bc;* thickness (*ld*) at base of thickened portion; polar diameter (*a*), measured by line *u*+line *m;* thickness (*ls*) at top of thickened portion. *A*, Outline of beet root, with lines and letters indicating measurements taken for computation of shape indices. The root illustrated has the following shape indices: *a*/*d*, 1.000; *u*/*m*, 1.000; and *ld*/*d*, 0.200. *B*, Outline of a flattened root having the lowest *a*/*d* value for an individual root in the material studied. The drawing represents a root having the following indices: *a*/*d*, 0.650; *u*/*m*, 1.000; and *ld*/*d*, 0.200. *C*, Outline of the longest individual beet in the material studied, having the following indices: *a*/*d*, 1.200; *u*/*m*, 1.000; and *ld*/*d*, 0.200. *D*, Outline of the most top-shaped individual root in the material studied, having the following indices: *a*/*d*, 1.000; *u*/*m*, 0.400; and *ld*/*d*, 0.200. *E*, Outline of most inverted top-shaped individual root in the material studied, having the following indices: *a*/*d*, 1.000; *u*/*m*, 1.500; and *ld*/*d*, 0.200. *F*, Outline representing a root having an *ld*/*d* index equal to the smallest value for this index in the roots studied. Indices: *a*/*d*, 1.000; *u*/*m*, 1.000; and *ld*/*d*, 0.100. *G*, Outline representing a root having an *ld*/*d* index equal to the largest value for this index in the roots studied. Indices: *a*/*d*, 1.000; *u*/*m*, 1.000; and *ld*/*d*, 0.300. *H*, Outline representing the most flattened root that could result from the extremes of indices for individual roots. The drawing is a composite of the extremes in indices that would contribute to a flat root. Indices: *a*/*d*, 0.650; *u*/*m*, 1.000; and *ld*/*d*, 0.100. *I*, Outline representing the longest root that could result from the extremes of indices for individual roots. The drawing is a composite of the extremes in indices that would contribute to a long root. Indices: *a*/*d*, 1.200; *u*/*m*, 1.000; and *ld*/*d*, 0.300.

but with a large, thick taproot. A beet having a small *ld/d* index (0.100) is shown in figure 1, *F*, and one with a large *ld/d* index (0.300) is shown in figure 1, *G*. A beet having a taproot of medium thickness, with an *ld/d* index of 0.200, is shown in figure 1, *A*; *A*, *F*, and *G* differ only in the *ld/d* index.

METHOD OF ANALYSIS

These studies were originally planned so that the data could be analyzed statistically by Fisher's analysis of variance method (*4*). However, drought, excessive rain, poor stands, and other factors made it impossible to record the data for a complete design as planned. Consequently, the results were not well adapted for analysis by the variance method. A study of the data indicated that in most cases comparisons could be made more satisfactorily by Student's method (*8*).

EFFECT OF VARIOUS FACTORS ON SHAPE

SIZE

For the study of the influence of size on the shape indices, twelve 100-root samples of large beets and twelve 100-root samples of small beets were harvested from 11 populations, as follows: One sample of large and one of small beets from each of the two plantings at Arlington Experiment Farm in 1933; from both light and heavy soil from the two plantings at Beltsville, Md., in 1933; from each of two plantings at Arlington Experiment Farm in 1934; and from plots on both heavy and light soil from the first planting at Beltsville in 1934; and two samples of large and two of small beets from the crop grown at Beltsville in 1935. In each case the large- and small-beet samples to be compared were harvested from the same population and had been grown as nearly as possible under the same environmental conditions.

The means for the two size samples from the same population were paired for statistical analysis by Student's (*8*) method. The mean diameters of the two size samples varied with the different populations; however, in each population the beets in the large-size sample had a mean diameter approximately 2 cm. greater than the mean diameter of the beets in the corresponding small-size sample. Reference to table 1 will show that the 12 pairs of samples used in this study of the influence of the size on shape of root were taken from populations grown under a wide range in soil texture, moisture supply, and season of planting and harvesting.

A summary of the data on the influence of size on the three shape indices, analyzed by Student's (*8*) method, is presented in table 2. The odds are 9,999 to 1 that beets of small equatorial diameter (*d*) have greater *a/d* and *ld/d* indices than those having a large diameter. The odds are 19 to 1, or barely significant, that roots of large equatorial diameter have greater *u/m* indices than roots having a small equatorial diameter.

These data show that the size of the root as measured by its equatorial diameter (*d*) must be taken into consideration in the study of variation in shape of beet roots.

TABLE 2.—*Influence of size on shape of beet roots*[1]

Index	Mean index value		Difference	Odds
	Large beets	Small beets		
a/d	0.905	0.936	0.031	[2] 9,999:1
u/m	.658	.631	.027	[3] 19:1
ld/d	.185	.215	.030	[2] 9,999:1

[1] 12 samples of large beets and 12 of small beets were compared in each test

[2] Odds that small beets have greater indices than large ones

[3] Odds that large beets have greater indices than small ones

SOIL TEXTURE

The 100-root samples taken for the study of the influence of soil texture on shape were all graded to size. All the populations were grown under conditions as nearly the same as possible, except that one lot of samples was taken from plots on heavy soil and the other lot of samples from plots on light, sandy soil. The samples had a mean diameter of approximately 6 cm. Ten pairs of means from 100-root samples were studied by Student's (*8*) method. A summary of the data is given in table 3.

TABLE 3.—*Influence of soil texture on shape of beet roots*[1]

Index	Mean index value		Difference	Odds[2]
	Heavy soil	Light soil		
a/d	0.968	0.941	0.027	61:1
u/m	.674	.632	.042	100:1
ld/d	.234	.198	.036	1,110:1

[1] 10 samples from heavy soil and 10 from light soil were compared in each test.

[2] Odds that heavy soil produces beets of greater *a/d*, *u/m*, and *ld/d* indices

The odds are 61 to 1 that beets when grown on heavy soil have greater *a/d* indices than when grown on light soil; 100 to 1 that heavy soil produces beets having greater *u/m* indices than beets of the same size grown on light soil; and 1,110 to 1 that the *ld/d* index is greater for beets grown on heavy soil than for those grown on light soil.

FERTILIZER

All of the material for the fertilizer studies was grown at Beltsville in 1933 and 1934. The 10 samples from each fertilizer treatment consisted of 1 sample from heavy and 1 from light soil from the 1933 crop, and 2 samples from the 2 soil types from both of the crops grown in 1934.

Representative samples were harvested from plots in each of the three fertilizer treatments in each of the crops studied. Ten 100-root samples from each of the three fertilizer treatments were used in pairing for statistical analysis.

In analyzing the data by Student's (*8*) method, the mean index for the 100-root samples from one fertilizer treatment was paired with that for a similar sample from the same crop but from a different fertilizer treatment. For each crop the mean index for 100 roots from treatment 1 was paired with that for 100 roots from treatment 2; the mean index for treatment 2 was then paired with that for treatment 3. Finally, the mean indices for samples from treatments 1 and 3 were paired, thus giving 3 sets of pairs for each of the 10 samples from each fertilizer treatment.

A summary of the data from the fertilizer studies is given in table 4. In no case was the difference due to the fertilizer treatment significant.

TABLE 4.—*Influence of fertilizer treatment on shape of beet roots* [1]

Index	Mean index value for treatment—			Treatments paired	Difference	Odds
	1	2	3			
a/d	0.934	0.927	0.914	1 and 2	0.007	3·1
				2 and 3	.013	11:1
				1 and 3	.020	18:1
u/m	.665	.657	.681	1 and 2	.008	1:1
				2 and 3	023	9:1
				1 and 3	.016	5:1
ld/d	.205	.209	216	1 and 2	004	2.1
				2 and 3	007	3:1
				1 and 3	011	4:1

[1] 10 samples from each of 2 fertilizer treatments were compared in each test

Less time was required for the beets on the heavily fertilized plots to reach a marketable size than on the less fertile plots. No doubt significant differences in shape indices could be shown between random samples harvested on the same date from the three treatment plots, resulting from the differences in growth rate caused by the treatments. However, the data in table 4 show that the indices for beets of the same size were little affected by the treatments.

SEASON

For the study of the influence of time of planting on shape, 100 roots of the same diameter were harvested from each of the four crops grown at the Arlington Experiment Farm in 1934 and from each of the four crops grown at Beltsville in 1935 and 1937. In 1934 and 1935 the 100-root samples were obtained by combining 4 samples of 25 roots each harvested from the same plots but from different parts of each plot. In 1937 a sample of 100 roots taken from the entire population was used. The 100-root samples were considered to be representative of the respective populations. It is obvious that great variation in climatic conditions may occur during the same season in different years. Data on the effect of season on root shape are available for only 3 years. Additional data would be needed to enable one to draw reliable conclusions as to seasonal influence on the shape of roots. While the seasonal data are too limited to justify general conclusions, they indicate that beet roots grown late in the season when the day length is becoming shorter and temperatures are declining have a greater length as determined by the *a/d* index than roots grown earlier in the season when temperatures are high and the day is longer.

The thickness of the taproot, as determined by the *ld/d* index, appears to be greatest in beets grown late in the season.

A summary of the data is given in table 5.

TABLE 5.—*Influence of date of planting on shape of beet roots*

Date of planting	Date harvested	Diameter	Mean shape index[1]		
			a/d	*u/m*	*ld/d*
		Cm.			
Apr. 9, 1934	July 3, 1934	5.52±0.007	0.896±0.009	0.594±0.009	0.181±0.004
May 9, 1934	Aug. 24, 1934	5.54± .038	.930± .010	.585± .007	.143± .005
June 7, 1934	Sept. 14, 1934	5.52± .056	.921± .012	.675± .010	.153± .004
Aug. 1, 1934	Oct. 12, 1934	5.53± .111	.954± .015	.758± .007	.160± .003
May 8, 1935	July 2, 1935	5.08± .027	.923± .015	.590± .005	.181± .009
June 4, 1935	Aug. 6, 1935	4.97± .015	.962± .011	.637± .011	.177± .005
July 9, 1935	Sept. 15, 1935	5.03± .031	.955± .008	.761± .007	.213± .007
Aug. 5, 1935	Oct. 11, 1935	4.98± .047	.994± .012	.743± .009	.272± .004
June 19, 1937	July 23, 1937	4.97± .027	.978± .010	.585± .009	.183± .010
July 19, 1937	Sept. 28, 1937	4.98± .030	.990± .013	.734± .007	.195± .005
July 30, 1937	Sept. 30, 1937	4.96± .028	1.082± .011	.719± .008	.245± .004
Sept. 23, 1937	Nov. 5, 1937	4.93± .045	1.113± .008	.629± .010	.300± .006

[1] Values represent mean index and probable error.

In 1934 the differences in the mean *a/d* indices for the four crops were not significant except between the first and fourth crops; in this case the difference was 3.3 times its probable error. The same condition was noted in 1935. The difference between the mean *a/d* indices for the first and fourth crops was the only difference too great to be attributable to chance alone. In 1937 there was a significant increase in the *a/d* index for each successive crop except the second. In all 3 years the first of the four crops had the smallest *a/d* index and the last crop the largest; the intermediate crops did not show a consistent trend, except in 1937.

Of the three indices the *u/m* were the least consistent. The first crop in 2 of the 3 years had the smallest *u/m* index; but the last crop did not have the largest. There appears to be a tendency for beets grown during the early part of the season to be more top-shaped (fig. 1, *D*) than roots grown later in the season.

Beets grown late in the season tend to have thicker taproots as indicated by the *ld/d* index than those grown earlier in the season. Except for the large *ld/d* index for the first crop in 1934, the trend from a small to a larger value was fairly consistent. With the exception of the first crop in 1934, the *ld/d* index for the last crop was much larger than for the first. The large *ld/d* index for the first crop in 1934 may be due to the fact that the planting date was a month earlier than the first crop in 1935, and over 2 months earlier than in 1937. The low temperature and short day when the first 1934 crop was developing may have contributed to the large taproots in this case. However, the trend of the *a/d* and *u/m* indices of this crop was not affected by early planting.

Although the data recorded show a trend toward larger values for all of the three indices as the date of planting is delayed, it should be emphasized again that, in view of the great differences in environmental conditions that may occur at the same time of year in different seasons, definite conclusions as to the influence of season on the shape indices cannot be drawn from only 3 years' data.

DISCUSSION

The analysis of the recorded data shows that there are significant differences in the shape of beet roots that can be attributed to environmental factors. Although the differences in mean indices for 100-root samples were in many cases shown to be statistically significant, the differences in shape were often so small that it was impossible to recognize them by visual inspection of the entire samples. The extremes in magnitude of the individual root indices found in the large number of individual roots studied are shown in figure 1, *B*, *C*, *D*, *E*, *F*, and *G*. All of the drawings shown in figures 1 and 2 represent beet roots having the same equatorial diameter (d). The values 1.000, 1.000 and 0.200 for a/d, u/m, and ld/d indices, respectively (fig. 1, *A*), were used as the basic values for all of the drawings except for figure 1,

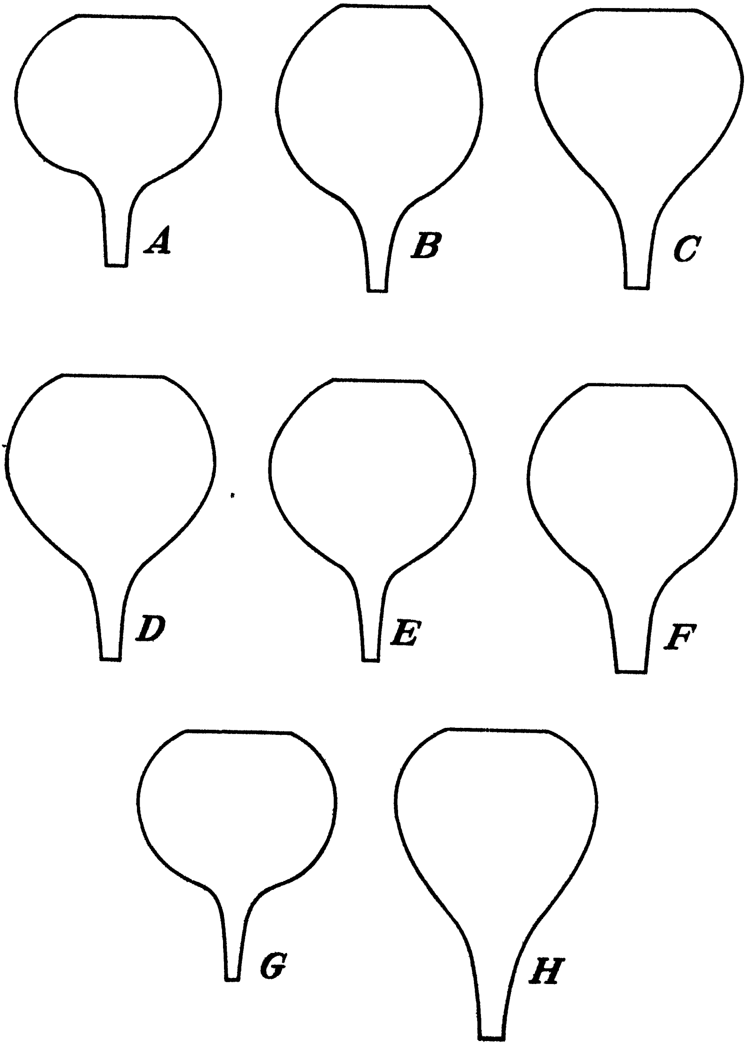

FIGURE 2.—FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE.

H and *I*, and figure 2, *G* and *H*. These values were kept constant except for one index in each drawing. Figure 1, *B* and *C*, indicates the extremes in *a/d* values for single roots in the many roots studied. The smallest *a/d* value for an individual root was 0.650 (*B*) and the largest 1.200 (*C*). Figure 1, *D* and *E*, shows the extremes in the *u/m* index for individual roots. The least *u/m* value for a single root was 0.400 (*D*) and the largest, 1.500 (*E*). The extremes in the *ld/d* index were 0.100 (*F*) and 0.300 (*G*).

The outline drawings *A* to *F* in figure 2 show the extremes in the mean indices for the 100-root samples. The smallest mean *a/d* index for the 100-root samples was 0.840 for a sample from the first crop at Arlington in 1933 (fig. 2, *A*), and the largest was 1.080 for 100 roots from the second crop on heavy soil at Beltsville in 1934 (*B*). The extremes in the *u/m* index for 100-root samples were 0.520 for a sample from the first crop at Arlington in 1933 (*C*) and 0.726 for a sample from the first crop on heavy soil at Beltsville in 1934 (*D*). The extremes in the *ld/d* index for the 100-root samples were 0.147 for the second crop on light soil at Beltsville in 1934 (*E*) and 0.279 for a sample from the crop on heavy soil at Beltsville in 1933 (*F*).

The outline drawings in figure 2, *G* and *H*, are composites showing the greatest variation in shape that could result from the extremes in mean indices for the 100-root samples. *G* represents the most nearly spherical beet that could result from the extremes in means for the three indices within the material studied. *H* indicates the longest root that could result from the extremes in mean indices for 100 roots.

It may be assumed that *G* and *H* in figure 2 represent somewhere near the extremes in mean shape for the 100-root samples that might be expected to result from this particular strain under the range of environmental conditions that prevailed during the 5 years of study. If every beet in a sample approached the shape of *G* in figure 2 and those in another sample approached the shape of *H*, it would not be difficult to recognize a difference in the mean shape of the two samples by visual inspection. With *G* and *H* representing the extremes in means for samples, it is obvious that the variation in all three indices that may occur in individual roots within a sample makes it prac-

EXPLANATORY LEGEND FOR FIGURE 2

Shape indices of beet roots. *A*, Outline representing a beet root having an *a/d* index equal to the smallest mean *a/d* index for a 100-root sample among the samples studied. Indices: *a/d*, 0.840; *u/m*, 1.000; and *ld/d*, 0.200. *B*, Outline representing a beet root having an *a/d* index equal to the largest mean *a/d* index for a 100-root sample. Indices: *a/d*, 1.080; *u/m*, 1.000; and *ld/d*, 0.200. *C*, Outline representing a beet root having a *u/m* index equal to the smallest mean *u/m* index for a 100-root sample. Indices: *a/d*, 1.000; *u/m*, 0.520; and *ld/d*, 0.200. *D*, Outline representing a beet root having a *u/m* index equal to the largest mean *u/m* index for a 100-root sample. Indices: *a/d*, 1.000; *u/m*, 0.726; and *ld/d*, 0.200. *E*, Outline representing a beet root having an *ld/d* index equal to the smallest mean *ld/d* index for a 100-root sample. Indices: *a/d*, 1.000; *u/m*, 1.000; and *ld/d*, 0.147. *F*, Outline representing a beet root having an *ld/d* index equal to the largest mean *ld/d* index for a 100-root sample. Indices: *a/d*, 1.000; *u/m*, 1.000, and *ld/d*, 0.279. *G*, Outline representing a beet root having indices equal to the three mean indices for 100-root samples that were most indicative of spherical-shaped roots. Indices: *a/d*, 0.840; *u/m*, 0.726; and *ld/d*, 0.147. *H*, Outline representing a beet root having indices equal to the three mean indices for 100-root samples that were most indicative of long-shaped roots. Indices: *a/d*, 1.080; *u/m*, 0.520; and *ld/d*, 0 279.

tically impossible in many cases to arrive at any estimate of the mean shape of a sample of root except by very careful measurement and the computation of indices. The comparison of samples by visual inspection is much more difficult if the samples consist of roots of various sizes, since it has been shown that the size of root is one of the most consistent factors influencing the index values.

The results of these studies indicate that significant differences in shape of beet roots may result from environmental causes. However, in a uniform strain of beets, such as that of Detroit Dark Red used in this work, the differences in shape indices for representative root samples may be very small except in cases where the crop is grown under wide extremes of soil, climatic, or seasonal conditions.

SUMMARY AND CONCLUSIONS

These studies included the influence of size of root, soil texture, soil fertility, and season of planting on the shape of beet roots.

Three indices of shape were computed for individual roots in samples of 100 roots each, harvested from 17 crops of the variety Detroit Dark Red, and grown under a wide range of environmental conditions over a period of 5 years.

All three of the indices were found to be influenced by the size of the roots. Small beets were longer and more top-shaped and had thicker taproots, in proportion to their equatorial diameter, than large beets.

Beets grown in heavy clay soil were longer and had thicker taproots, n relation to their equatorial diameter, than roots grown in deep sandy soil.

Wide extremes in soil fertility, as judged by the rates of fertilizer application, failed to produce significant differences in any of the three shape indices for roots of the same size as determined by the equatorial diameter.

Although the data on the influence of season of planting are limited, they indicate that the roots from crops planted in late summer tended to be longer, and thicker at the plane *ld*, than those from crops grown in the spring and early summer.

While the differences in the shape indices for samples of 100 roots grown under different environmental conditions were found to be statistically significant, the differences between means were in many cases very small.

Variation in the shape of individual roots within a sample was too great to permit a satisfactory estimate by visual inspection of the shape of a representative sample from a population.

It is concluded from these studies that the shape of roots of a good strain of beets of the variety Detroit Dark Red may be altered by environmental conditions and that these variations can be shown by numerical shape indices. However, variations in shape are not great except when the crops are grown under wide extremes of soil, climatic, and seasonal conditions.

Difference in size of the individual roots composing the sample seems to be the most consistent cause of the differences in shape that appear to exist between random samples from populations grown under different environmental conditions. No satisfactory study of environmental influences on the shape of beet roots can be made on random samples unless the size of the individual root is taken into account.

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RELATIVE INFLUENCE OF THE VARIOUS FACTORS THAT AFFECT EGG PRODUCTION OF TURKEYS DURING DIFFERENT PARTS OF THE FIRST LAYING YEAR[1]

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INTRODUCTION

In a previous paper (*1*)[3] the writer showed the influence of various factors on first-year egg production in turkeys. These factors, in the order of their importance, were date of last egg (persistency), pauses (total length of all 7-day broody and nonbroody pauses), date of first egg (sexual maturity), and rate measured by percent production, exclusive of pauses, during March and April.

Egg production during the season when hatching eggs are wanted is of much greater practical importance than later in the year. The demand for hatching eggs does not come during a fixed period, however, since it varies from year to year and also to some extent in different geographic areas. In California most of the eggs are set before the end of April, but in some years eggs are set in May and even June. The price paid for hatching eggs usually decreases as the hatching season advances. The greater value of early eggs as compared with those laid late in the season suggested the need for information regarding the factors that influence egg production in different periods of the first laying year. A statistical analysis of turkey egg records was accordingly undertaken to determine the relative influence of the various factors previously correlated with total first-year egg production on egg production during different parts of the first laying year. This paper presents the results of that analysis.

MATERIALS AND METHODS

The data are for the same birds used by the writer (*1*) to determine the influence of different factors on total first-year egg production. The various measures of the factors correlated with egg production have already been described. It is sufficient here to recall that (1) date was in each case coded and stated as the number of days from the first of the year (in the case of date of hatch this was the number of days from the first of the year in which the bird was hatched; in the case of date of first egg and date of last egg it was the number of days from the first of the year in which the record was made, and this was in each case the year following the one in which the bird was hatched); (2) pauses were all periods of nonproduction of 7 or more days regardless of whether or not the bird became broody; (3) net rate was the percent production while laying, pauses of 7 or more days being excluded when calculating net rate. Net rate for May

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[2] The writer is indebted to Ward S Watson, who performed most of the computations, and to the Works Progress Administration, under whose auspices part of the tables were checked.

[3] Italic numbers in parentheses refer to Literature Cited, p 753.

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and June and after the end of June was in each case calculated for birds that laid six or more eggs in the given period, which reduced the sample to less than the total number of records used for other calculations.

The first laying year was divided arbitrarily into three periods (A) to the end of April, (B) May and June, (C) after the end of June (table 1). The means and standard deviations for first-year egg production, for the date of hatch, and for the various factors based on the first laying year have been presented elsewhere (*1*), together with the net spring rate for March and April. The means and standard deviations for egg production, number of pauses, length of pauses, and net rate in each of the periods considered in this paper are presented in table 1. Net rate for March and April was used for the period to the end of April. It will be observed that egg production decreased in each successive period, but since the periods were not of equal length no particular significance can be attached to this finding. The net rate also decreased. The number of pauses per bird showed a tendency to increase slightly after the end of June, but the length of pauses per bird varied irregularly.

Zero order coefficients of correlation were calculated from grouped data. Sheppard's correction was not applied. Coefficients of multiple correlation were calculated by the method of Wallace and Snedecor (*8*). Formulas for all other calculations used are described by Ezekiel (*3*).

TABLE 1.—*Mean and standard deviation for egg production per bird, for number and length of pauses per bird, and for net rate, during different parts of the first laying year; 1934–36*

Period and year[1]	Egg production		Pauses		Length of pauses		Net rate	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
A, to the end of April:	*Number*	*Number*	*Number*	*Number*	*Days*	*Days*	*Percent*	*Percent*
1934	36.1	13.4	0.7	0.7	13.9	19.2	[2]71.1	5.8
1935	40.2	15.3	.5	.7	14.0	11.8	[2]68.8	9.6
1936	38.7	13.9	.7	.7	20.2	23.3	[2]99.1	9.6
B, in May and June								
1934	24.1	13.0	.6	.8	12.6	15.7	59.6	9.0
1935	23.4	11.5	1.1	.9	22.0	14.3	61.8	10.1
1936	25.2	13.3	.6	.7	10.7	12.6	63.5	9.8
C, after the end of June								
1934	18.0	15.2	1.1	1.2	16.5	16.8	52.3	9.7
1935	17.1	9.9	.8	1.1	14.5	17.4	53.5	14.4
1936	15.6	12.3	.8	1.1	11.7	14.8	57.9	9.5

[1] The total number of birds for which records were available was as follows. 1934, 91, 1935, 74, 1936, 73.
[2] Spring net rate for March and April only.

STATISTICAL ANALYSIS

The zero order coefficients of correlation for egg production during the first laying year and during parts of the first year were computed, and thev alues of $\bar{r}$, the coefficient most probably representing the true value for the universe from which the sample was drawn, are given in table 2. There was a high correlation between total egg production and egg production during any part of the year. This correlation is, of course, partly spurious because the whole is correlated with a part. There is, however, a highly significant correlation between egg production to the end of April and the egg production in May and June. The correlation between egg production before the end of April and after the end of June is low and of doubtful significance. Egg production in May and June is apparently correlated with egg production after the end of June, but the coefficients vary from a low value in 1934 to highly significant values in 1935 and 1936. The low and variable egg production after the end of June indicates that egg production in this period is adversely affected by hot weather. This is reflected in reduced egg production and increased variability of egg production after the end of June (table 1).

TABLE 2.—*Zero order coefficients of correlation—number of eggs laid during the first year and during different parts of the first laying year—values of $\bar{r}$, 1934–36*

Egg-production periods correlated	1934	1935	1936
Year, to end of April	0 751	0 813	0 692
Year; May and June	751	806	813
Year, after the end of June	650	692	627
To end of April, May and June	474	508	399
To end of April; after the end of June	170	275	000
May and June, after the end of June	163	455	338

TABLE 3.—*Zero order coefficients of correlation—values of $\bar{r}$—for various factors correlated with egg production in different parts of the first laying year, 1934–36*

Variables correlated with egg production in periods A, B, and C	A, egg production to the end of April			B, egg production during May and June			C, egg production after June		
	1934	1935	1936	1934	1935	1936	1934	1935	1936
Date of hatch	-0 228	-0 259	0 000	-0 157	-0 128	0 170	0 000	-0 269	0 106
Age at first egg	- 403	- 425	- 567	000	000	- 093	000	000	000
Date of first egg	- 791	- 757	- 655	- 109	228	000	.000	.000	000
Age at last egg	320	380	000	383	.478	296	727	705	.805
Date of last egg	262	324	000	347	.504	.355	.768	728	859
Length of laying year	413	578	261	326	.458	375	746	615	812
Rate (year)	000	144	- 060	- 090	327	237	.000	000	.000
Spring rate (March and April)	170	268	140	059	385	292	194	284	.213
Rate in May and June	000	000	- 032	097	526	289	.257	381	000
Rate after June	-.209	- 108	- 119	- 294	000	000	054	- 105	-.142
Pauses:									
Number (year)	000	- 044	- 171	000	-.088	-.132	210	000	206
Length (year)	- 198	- 342	- 508	- 419	- 296	- 526	178	000	.186
Number to end of April	- 226	- 504	- 350	- 365	- 441	- 246	- 082	-.142	000
Length to end of April	- 459	- 557	- 557	- 467	-.475	- 506	044	-.152	.053
Number in May and June	000	.000	000	- 090	- 136	- 187	000	.000	-.039
Length in May and June	000	- 135	- 062	- 370	- 163	-.310	.139	.000	- 042
Number after June	176	152	000	112	191	000	.323	.210	.372
Length after June	000	166	.000	000	139	000	.051	000	.300

Table 3 gives the values of $\bar{r}$ for the various factors correlated with egg production in different parts of the first laying year. Egg production to the end of April was correlated with date of and age at first egg, the coefficient of correlation being higher for date of first egg than for age at first egg. The coefficients of correlation for age and date of last egg with egg production to the end of April were low in 1934 and 1935, and nil in 1936. Length of laying year was also correlated with egg production in this period, but the coefficient is less than for date of first egg. Length, but not number, of pauses during the year is correlated with egg production to the end of April, whereas rate during the year is not. Number of pauses and length of pauses to the end of April and also net spring (March and April) rate are all correlated with egg production in this period and the coefficients are all higher than the corresponding correlations calculated from data for the year as a whole. These three factors for the two later periods (May and June, after June) are not correlated with egg production to the end of April. To summarize, egg production to the end of April is correlated with sexual maturity (particularly as measured by date of first egg), with number and length of pauses to the end of April, and with length of pauses during the year, with net spring rate, and, except in 1936, with persistency (date or age at last egg).

Egg production in May and June is correlated with persistency (date of or age at last egg), and with length of pauses for the year and for the first two periods but not the last period (after June). Egg production in this period is also correlated with net spring rate and with net rate in May and June in the years 1935 and 1936, but in neither case is the coefficient for the 1934 data significant. These results, while not conclusive, indicate that net rate prior to the end of June usually has some influence on egg production in May and June. Date of or age at first egg (sexual maturity) and number of pauses are not correlated with egg production in this period (table 3).

Egg production after the end of June (table 3) is correlated with age at and date of last egg and with net spring (March and April) rate, and, of course, also with length of laying year. The correlation with other factors is nil or of doubtful significance. The high correlation with length of laying year is to be expected since length of laying year is determined by date of first and last egg (or age at first and last egg). The positive correlation with number of pauses after the end of June is probably partly due to the inclusion of birds that did not lay in this period and their classification as nonpausers.

The coefficients of multiple correlation R and $\overline{R}$, the most probable values for the universe from which the sample was drawn, were determined for egg production in each of the three periods and the four factors previously used in a study of first-year egg production (*1*), namely, date of first egg, length of pauses during the year, net spring (March and April) rate, and date of last egg. It is evident from the values of $\overline{R}$ presented in table 4 that these factors accounted for nearly all the variance in egg production to the end of April but that they did not fully account for variance in May and June, nor after June. Date of first egg has the greatest influence on egg production to the end of April; the other factors appear to have a lesser influence, but, nevertheless, in each case increase the coefficient of multiple correlation slightly. Egg production after the end of June is apparently determined mostly by date of last egg (persistency).

In order to find more precisely what the relative importance of these four factors was in determining egg production in the three periods under consideration, the coefficients of partial determination were calculated. These are presented in table 5. This table shows the influence on egg production in each of the three periods of the same four factors used in table 4—date of first egg, length of pauses during the year, net spring (March and April) rate, date of last egg.

The data in table 5 show that date of first egg has the greatest influence on egg production to the end of April. The order of importance for the other factors is length of pauses, date of last egg, and spring rate.

TABLE 4.—*Coefficients of multiple correlation—values of R—for various factors correlated with egg production in different parts of the first laying year; 1934–36*

Variables correlated with egg production in periods A, B, and C	A, egg production to end of April			B, egg production in May and June			C, egg production after June		
	1934	1935	1936	1934	1935	1936	1934	1935	1936
Date of first egg; date of last egg	0 809	0 774	0 654	0 349	0.514	0 338	0.785	0.725	0 862
Date of first egg; length of pauses	826	815	786	.469	361	522	.164	.000	.152
Date of first egg; rate	811	847	696	121	481	.270	180	.301	.183
Date of last egg; length of pauses	476	.398	.564	.797	.733	.836	787	.818	.869
Date of last egg; rate	286	385	.074	338	.587	412	.770	.745	.858
length of pauses, rate	245	398	.528	471	.446	.602	.248	.262	.286
Date of first egg, date of last egg; length of pauses	.893	.873	.837	800	731	.835	805	.824	.871
Date of first egg; date of last egg; rate	.825	.850	.691	340	.612	.398	787	.741	.862
Date of first egg; length of pauses;	853	.879	.820	490	520	.597	238	.279	.274
Date of last egg; length of pauses; rate	.495	.605	.567	.798	750	846	.790	819	.868
Date of first egg; date of last egg; length of pauses; rate	909	.908	852	801	.744	845	809	.821	870

TABLE 5.—*Influence on egg production in different parts of the first laying year of the four factors used in table 4*

[Reduction in unexplained variance]

Variables correlated with egg production in periods A, B, and C	Factors already considered	A, egg production to end of April			B, egg production in May June			C, egg production after June		
		1934	1935	1936	1934	1935	1936	1934	1935	1936
Date of first egg	Date of last egg; length of pauses	0 739	0 718	0.559	0 011	0 000	0.000	0 076	0 028	0.015
Date of last egg	Date of first egg, length of pauses	365	.293	.214	.538	.464	.585	638	.681	.753
Length of pauses	Date of first egg; date of last egg	414	.409	.476	589	.367	.659	083	.323	.063
Date of first egg	Date of last egg, rate	.651	.675	.475	.002	.047	.000	064	006	027
Date of last egg	Date of first egg, rate	.063	.017	.000	.103	.187	.092	.607	.503	734
Rate[1]	Date of first egg; date of last egg	072	.309	.088	.000	151	.050	.010	048	.000
Date of first egg	Length of pauses; rate	710	.729	.545	.023	.089	.000	.000	.010	.000
Length of pauses	Date of first egg; rate	.201	.191	.304	229	.051	.306	.025	.000	.043
Rate[1]	Date of first egg; length of pauses	142	.320	.141	.026	.161	.115	.031	.078	.053
Date of last egg	Length of pauses; rate	197	.247	058	.534	.454	.554	.600	.646	.732
Length of pauses	Date of last egg; rate	.178	.256	.317	591	.333	.658	.077	260	.068
Rate[1]	Date of last egg; length of pauses	024	.246	.003	0067	.054	.056	.014	.001	.000
Date of first egg	Date of last egg; length of pauses, rate	769	.722	.597	.012	000	.000	.078	.012	.015
Date of last egg	Date of first egg; length of pauses; rate.	362	227	.165	.529	.388	555	.633	.647	.738
Length of pauses	Date of first egg; date of last egg; rate	.456	.364	.475	.595	285	.659	090	.279	057
Rate[1]	Date of first egg; date of last egg; length of pauses	.138	257	088	007	040	051	017	000	.000

[1] Stands for net spring (March and April) rate

Egg production in May and June is apparently influenced about equally by length of pause and date of last egg. Spring rate and date of first egg have relatively much less influence on egg production in these months (table 5).

Of all the factors considered, date of last egg (persistency) has by far the most important influence on egg production after June (table 5). The other factors appear to have little influence on egg production after June, although the values for length of pause tend to be higher than those for date of first egg and spring rate.

DISCUSSION

The data presented indicate that the various factors which were correlated with egg production are not of equal importance in determining egg production in different parts of the first laying year.

Thus sexual maturity (date of first egg) is the most important factor influencing egg production to the end of April, whereas its influence on egg production is slight in May and June and negligible after the end of June. On the other hand, persistency, as measured by date of last egg, has greater influence than any other factor on egg production after June, an important influence on egg production in May and June, but relatively little influence on egg production prior to the end of April. Length of pauses has an important influence on egg production in the first two periods, and the data presented indicate that there is also some influence on egg production in the last period, after the end of June. These results thus confirm the earlier findings that the time factors have an important influence on first-year egg production but they show that, except perhaps for pausing, their influence is not exerted equally throughout the year. Sexual maturity and persistency have previously been shown to be independent variables in turkeys (*1*) and in chickens (*4*). The data presented in this paper show that there is little overlapping of their influence on egg production in the different periods, with sexual maturity influencing egg production early in the year while persistency influences egg production more later in the year.

Net spring rate appears to have less influence on egg production than sexual maturity, pauses, and persistency.

Net spring rate is positively correlated with egg production in each of the three periods, although some of the coefficients are low. The coefficients of correlation for net rate in May and June and egg production in May and June are above those for net spring rate and egg production in May and June except in 1936. Nevertheless, table 3 shows that net spring rate is more consistently positively correlated with egg production in the different periods than net rate for May and June or for after June. It has previously been shown that net spring rate is more closely correlated with first-year egg production than is net rate calculated for the year (*1*). From this evidence it may be concluded that net spring (March and April) rate is a better measure of rate than net rate calculated for the entire year, for May and June, or for after June.

Both number and length of pauses in the first two periods are negatively correlated with egg production in these periods. These factors for the first period are more closely correlated with egg production in the first two periods than those based on the entire year or on the two later periods. After the end of June, egg production tends to be positively correlated with length and number of pauses. Birds that lay more than a few eggs after the end of June almost invariably pause. Since, as stated above, birds that did not lay in this period or laid only a few eggs and then stopped, were classified as nonpausers, a positive correlation is to be expected. In this connection it should be recalled that length of pauses for the year is correlated with persistency (date of last egg). No particular significance can be attached to the positive correlation between length of pauses and egg production after June. The correlation is probably the result of an unfavorable environment which causes low egg production after June. (See previous discussion and Asmundson and Lloyd (*2*).) The low egg production after June may also result from the tendency to lay for only a short period, which is characteristic of some species. (See Rowan (*6*) and others.) This can only be a minor factor, however, since young turkey hens lay late in the summer and some of these lay in the autumn. There is good evidence to show that temperature has little influence on the initiation of egg production in turkeys (*7*). Once egg production has started, however, unfavorable temperatures may cause a cessation in egg production. High temperatures in July and August are probably one of the causes of low egg production after June. The apparently great influence of the unfavorable environment on egg production after June may account for the low correlation of egg production in this period with length of pauses and net rate. The relatively low correlation between egg production after June and egg production in the earlier periods may also be due to differences in the weather in the three periods.

The relatively low correlation of egg production after June with spring egg production, together with the relatively slight influence of persistency on spring egg production, would indicate that the practical turkey breeder could ignore egg production after June but should select for the three factors that influence spring egg production most. These, in the order of their importance, are: (1) Sexual maturity, which is best measured by date of first egg; (2) pauses, absence of pauses being desirable; and (3) net rate. Although net rate appears to be the least important of these, it would seem well worth while to select for improved rate in view of the variation shown to occur in this character in this paper (table 1) and by Marsden (*5*).

SUMMARY

The first laying year of turkey hens trap-nested in 1934, 1935, and 1936 was arbitrarily divided into three periods; (A) From beginning of egg production to the end of April; (B) May and June; and (C) after June. Egg production in each of these periods was correlated with various factors.

Sexual maturity (date of first egg) was found to be the most important factor influencing egg production to the end of April, the other factors in the order of their importance being length of pauses, date of last egg (persistency), and net rate.

Egg production in May and June was influenced most by length of pauses and date of last egg, whereas date of first egg and net spring rate had less influence on egg production in this period.

Persistency had by far the greatest influence on egg production after June while the coefficients for egg production with the length of pauses were low. Date of first egg and net spring rate had little or no influence on egg production in this period.

Persistency has little influence on egg production early in the year; hence the factors of greatest practical importance in selecting for improved egg production during the breeding season are, in the order of their importance, sexual maturity (date of first egg), length and number of all broody and nonbroody pauses, and net spring rate.

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INTERPRETATION OF MENDELIAN CLASS FREQUENCIES[1]

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INTRODUCTION

The application of the chi-square test to Mendelian class frequencies constitutes a familiar illustration of the general problem of testing a hypothesis by means of observed data. Since the test is almost universally employed by geneticists, it appears desirable to present a few principles that may serve as a guide to its interpretation.

The investigator should have no illusions regarding the ability of the test to prove or disprove the hypothesis under consideration. No such test exists. Neither should he harbor the mistaken idea that the test measures the probable truth of the hypothesis. Attempts to measure the probable truth of a hypothesis have always become involved in the controversial subject of inverse probability and have not been generally accepted by mathematical statisticians. The philosophy underlying the chi-square test is foreign to such a concept and avoids the difficulties to which it leads, but only at the expense of providing the investigator with a test that falls short of what is actually desired. The test merely shows whether or not the observed data are likely to be obtained under conditions of random sampling if the hypothesis under consideration is true. The investigator's conclusions in regard to the truth or falsity of the hypothesis can be nothing more than inferences based on the results given by the test and such other information relative to his particular problem as may be available.

THE CONCEPT OF RANDOM VARIATION

It is regrettable that the small amount of information which the chi-square test is capable of contributing to problems involving the testing of hypotheses has in the past been still further restricted by the failure of some investigators to comprehend the concept of random variation and its implications in experimental work. At least a part of the blame for this failure must be borne by the statistician, for the older chi-square tables are not adequate for an efficient application of the chi-square test. These tables tend to create the impression that only values of chi square larger than one would expect to arise by chance should be regarded as lying outside the normal range of variation.

The reason for this impression is a subject for speculation. One can hardly suppose that such statisticians as Pearson (*7*) [2] and Elderton (*1*) had any mistaken ideas as to what constitutes random variation, although it appears unlikely that any particular importance would

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[2] Italic numbers in parentheses refer to Literature Cited, p. 759.

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have been attached to a small value of chi square at a time when the test was used principally to measure the goodness of fit of frequency curves. If any objection to too good a fit occurred to them, they appear to have made no mention of it. Fisher (*3*) was fully aware of the implications of unusually good agreement between observation and theory, but proposed no better criterion for determining the normal range of variation of chi square than was provided by Elderton's table. This circumstance may have been due to the lack of a suitable criterion for establishing limits of variability in the case of skew frequency distributions.

In order to establish a criterion that will show whether or not an observed value of chi square is within the normal range of variation, it is necessary to arrive at an agreement as to what constitutes the normal range of variation. The senior author has found that the shortest range of values which will include 95 percent of the observations may be regarded as the normal range of variation, and has computed the following chi-square table (table 1):[3]

TABLE 1.—*Critical values of chi square for 5-percent level of significance*

Degrees of freedom	Value		Degrees of freedom	Value		Degrees of freedom	Value	
	Lower	Upper		Lower	Upper		Lower	Upper
1	0.000	3.841	11	2.954	20.305	21	9.267	33.921
2	.000	5.991	12	3.516	21.729	22	9.958	35.227
3	.003	7.816	13	4.099	23.135	23	10.656	36.526
4	.085	9.530	14	4.700	24.525	24	11.361	37.818
5	.298	11.071	15	5.318	25.900	25	12.073	39.103
6	.607	12.802	16	5.948	27.263	26	12.791	40.383
7	.990	14.369	17	6.591	28.615	27	13.514	41.658
8	1.425	15.897	18	7.245	29.955	28	14.243	42.927
9	1.903	17.393	19	7.910	31.285	29	14.977	44.192
10	2.414	18.861	20	8.581	32.607	30	15.716	45.451

The principle on which table 1 is based is theoretically sound and lends itself well to the problem of establishing limits of variability for a large variety of statistics that are distributed according to skew frequency curves. It so happens that the ordinates of the chi-square distribution at the two extremities of this range are equal when the number of degrees of freedom is greater than 2. This property, which can be demonstrated mathematically, is the necessary and sufficient condition that a particular value of chi square regarded as lying outside the normal range of variation cannot have a probability of occurrence equal to, or greater than, the probability of occurrence of some value on the other side of the mode that would be accepted as being within the normal range of variation. For 1 and 2 degrees of freedom, the lower bound of admissible values of chi square lies at zero, which agrees with the known fact that observed values in the neighborhood of zero are expected to occur more frequently than any other. For an extremely large number of degrees of freedom, the chi-square distribution approaches a symmetrical form resembling the normal curve of errors. In such a case, the normal range of variation, as defined in this paper, would exclude approximately 2.5 percent of the values at each extremity of the distribution, a phenomenon that has a well-known parallel in the usual treatment of the normal curve. However, this condition is not realized until the number of degrees of freedom becomes very large. For 30 degrees of freedom, the criterion presented in this paper excludes only 1.5 percent of the values of chi square at the lower extremity of the curve and 3.5 percent of the values at the upper extremity.

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[3] Acknowledgment is made for the assistance of Kate W. Robey, of the Bureau of Animal Industry, in the construction of this table.

It is evident that the table leads to results that are perfectly consistent with the nature of the frequency distributions involved for various numbers of degrees of freedom. A similar method of approach has recently been used by Fertig and Proehl (*2*) in a consideration of Neyman and Pearson's likelihood function. In fact, the table that Fertig and Proehl (*2*) have presented for testing an observed standard deviation may be used to test an observed value of chi square in accordance with the theory embodied in Hendricks and Robey's table. For an observed value of chi square, one may compute Fertig and Proehl's k by means of the formula

$$k=0.434294\,\frac{\chi^2}{n-2}-\log_{10}\frac{\chi^2}{n-2},$$

in which n is the number of degrees of freedom involved, and determine the probability associated with that value of k by deducting 2 units from the correct number of degrees of freedom before entering the table.

Table 1 and the table presented by Fertig and Proehl (*2*) provide more satisfactory criteria than have previously been available for deciding whether or not an observed value of chi square, as stated by Pearson (*7*), "is such that it can be reasonably supposed to have arisen from random sampling."

An investigator obtaining a value outside the normal range of variation should reconsider his hypothesis and subject his data to further scrutiny. The conclusions to be drawn in the case of a value larger than one would expect to arise by chance, are familiar to most geneticists, but the interpretation to be placed on a value smaller than one would expect may not be so apparent. Such a value does not necessarily imply either that the hypothesis is wrong or that there is something wrong with the experimental technique, for unusual sets of data occasionally arise by chance. However, any investigator who consistently obtains values of chi square smaller than would be expected to arise by chance should infer, in the case of Mendelian class frequencies, that he is working with data that were consciously or unconsciously selected to agree with the hypothesis under consideration.

DETECTION OF SELECTED DATA

In some cases, unusually close agreement between observation and theory occurs because of a natural tendency on the part of the investigator to stop his experiment when the data seem to agree with his hypothesis. The fact that such a procedure results in selected data may not have occurred to some geneticists. In this connection, it may be pointed out that, according to the law of chance, an investigator may obtain data that will satisfy any one of several different hypotheses regarding the proportion of observations to be expected in each of the various classes. If he stops his experiment when the results seem to agree with his chosen hypothesis, his experiment cannot be regarded as a fair test of the hypothesis.

Data derived from such an experiment generally yield unusually small values of chi square because of the tendency of investigators to underestimate the amount of variability that can be accepted as arising by chance. This fact is illustrated in some data reported by Quinn and Godfrey (*8*). These data, considered en masse, consist of 44 classes of observations in which the method of selection just referred to is known to have been used. The 44 classes yield 22 degrees of freedom for which chi square has the value 6.398. By referring to table 1 it will be observed that the normal range of variation of chi square for 22 degrees of freedom is 9.958 to 35.227. The small value of chi square obtained from the data, therefore, reflects the artificial agreement between observation and theory that was imposed by the selection of the data. However, in this case, supplementary evidence is available to confirm the conclusions reported and there is no question of their validity.

The older literature in the field of genetics contains reports of data that seem to have been obtained under similar conditions. For example, Lippincott (*6*) reported three sets of data obtained from backcross matings in a study of plumage-color inheritance in chickens. From the use of only the totals in his tables, the three sets of data yield 7 degrees of freedom for which chi square has the value 0.550. The normal range of variation of chi square for 7 degrees of freedom is 0.990 to 14.369. In this illustration, there is also no question of the validity of the conclusions reported. However, such selected data are undesirable and may at times retard the discovery of genetic relationships.

A critique of Mendel's classic work recently reported by Fisher (*4*) shows how selected data may appear to substantiate an incorrect conclusion. Fisher (*4*) found unusually close agreement between observation and theory in Mendel's data. For 84 degrees of freedom, chi square had the value 41.6. In order to determine the normal range of variation, one may make use of the approximation to the chi-square distribution for large numbers of degrees of freedom suggested by Fisher (*3*). The range is 60 to 111. Therefore, the observed value, 41.6, is smaller than one would expect under conditions of random sampling and indicates that the data are a selected set. In one phase of his work, Mendel's data agree closely with the 2 : 1 ratio which he expected to obtain, but differ significantly from the correct ratio.

One other example may be cited because it illustrates another method of detecting selected data. Smith (*9*) reported data on the inheritance of annual versus biennial growth habit in sweetclover plants and concluded that the 2 factors segregated in a 3:1 ratio in the F_2 generation. The totals of his 2 classes of observations, obtained from 91 families, yield 1 degree of freedom for which chi square has the value 2.592. This result is within the normal range of variation. 0.000 to 3.841, and appears to substantiate his hypothesis. However, when the chi square test is applied to the totals of 9 subgroups of his observations, 8 containing the data for 10 families each and 1 containing the data for the remaining 11 families, chi square has the value 42.345. The normal range of variation of chi square for 9 degrees of freedom is 1.903 to 17.393. This result indicates that the data are more variable than would be expected if the hypothesis were correct and that the agreement of the totals with the hypothesis, as reported by Smith (*9*), is spurious. The same conclusion in regard to this set of data has been reported by Kirk and Immer (*5*), who made a study of the results reported for individual families.

REPORTING DATA ON POULTRY GENETICS

Poultry geneticists, as a group, apparently do not report data that agree too closely with the hypotheses tested. Warren[4] has summarized a large amount of data obtained by various investigators studying autosomal factors in the fowl.[5] Omitting data for factors involving linkage, the summary yields 213 degrees of freedom for which chi square has the value 284.4. The normal range of variation is 174 to 255. The observed value of chi square is thus larger than one would expect, but a careful examination of the data indicates that this is due to the presence of a few highly discordant observations rather than to any systematic bias in the data.

An investigator need not hesitate to report data in which the agreement between observation and hypothesis differs significantly from what is expected under conditions of random sampling if he is convinced that the discrepancy is due to chance. To avoid criticism, an investigator may be tempted to report only such results as appear to be favorable to his hypotheses and to withhold results that exhibit either less or more variability than is expected. Such a procedure, if practiced consistently, will inevitably lead to an accumulation of fictitious data that accomplishes little more than to reflect the personal opinions of the investigators who obtained it.

SUMMARY

The principles involved in the interpretation of Mendelian class frequencies are discussed. The application of the chi-square test to such data is considered as a means of detecting data which do not agree with what is expected under conditions of random sampling. A new chi-square table, which appears to be more useful for this purpose than the tables in use at the present time, is presented.

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EXPERIMENTAL TRICHOSTRONGYLOSIS IN SHEEP AND GOATS[1]

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INTRODUCTION

In 1918 Waterman (*14*)[2] described a disease occurring in goats on the island of Curaçao in the Dutch West Indies, the disease being characterized by weakness, emaciation, and profuse diarrhea. Waterman found that this disease often resulted in the death of the animal, and he considered the symptoms to be due to malnutrition combined with an infestation of small trichostrongyles, identified as *Trichostrongylus instabilis* (= *T. colubriformis*). According to Mönnig (*10*), who later (*11*) described the clinical symptoms and gross pathology of this disease as it occurred in South Africa, it was not until 1923 that these small trichostrongyles were recognized as the definite cause of serious losses among sheep in that part of the world. At about the same time, the nematodes in question were also suspected of causing severe losses among sheep in California.[3] In 1932 Boughton (*4*) described typical symptoms of trichostrongylosis in connection with an outbreak of the disease in goats in Texas, and in 1937 Schmidt (*12*) reported briefly on unsuccessful attempts to treat the disease. That trichostrongylosis is also of considerable economic importance in Australia has been shown by Edgar (*5*), Kauzal (*9*), Bennetts (*3*), and Gordon and Ross (*8*), who studied various clinical aspects of the disease, and by Seddon and Ross (*13*), Belschner and Edgar (*2*), Gordon (*7*), and Gordon and Ross (*8*), who worked on the treatment for it. So far as the writer is aware, the paper by Gordon and Ross is the only one containing a record of the production of fatal cases of trichostrongylosis by the administration of infective larvae to susceptible host animals.

The purpose of the present paper is to report observations on cases of trichostrongylosis produced in host animals raised in cages relatively free from parasites and experimentally infected with known numbers of infective larvae of *Trichostrongylus* spp.

EXPERIMENTAL METHODS

Eleven cross-bred Hampshire-Southdown lambs and three Saanen kids, about 6 months old, were used in this investigation. These host animals were born at the Agricultural Research Center, Beltsville, Md. They were kept at all times in cages having concrete floors, which were cleaned daily to prevent infestation with parasites other than those administered.

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[1] Received for publication November 3, 1938.

[2] Italic numbers in parentheses refer to Literature Cited, p. 769.

[3] Personal communication by the late M. C. Hall, formerly Chief of the Zoological Division, Bureau of Animal Industry.

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The experiments involving lambs 57, 58, 68, 70, 72, 122, and 61 were conducted in 1935. The first five lambs of this group received larvae of *Trichostrongylus colubriformis*, whereas lamb 61 remained uninfected and served as a control. The experiments involving lambs P2, 112, 117, and 123 and kids 127, 130, and 131 were conducted in 1937. These animals received larvae of *T. vitrinus*, *T. colubriformis*, and a small number of the larvae of *T. axei*.

To obtain infective larvae, specimens of the nematodes desired were removed from infested sheep at the time of slaughter, and the females of each species were isolated and cut up with a pair of fine scissors. The eggs thus liberated were cultured at room temperature for about 7 days in sterile sheep feces and animal charcoal. The infective larvae were removed from the cultures by means of the Baermann apparatus and suspended in water. They were then administered by means of a funnel and rubber tube to parasite-free lambs, which later served as sources of infective material for the experiments. When these lambs began to pass trichostrongyle eggs, the feces were cultured as indicated above, and the infective larvae obtained were administered to the experimental animals as shown in tables 1 and 2.

The progress of the infestations was followed by the use of standard egg-counting methods, and the data thus obtained were recorded in terms of the number of eggs per gram of feces. The general appearance of the animals and the consistency of the feces were noted from day to day. Samples of blood were taken from all the animals at frequent intervals, and the total number of red and white cells per cubic millimeter of blood, the amount of hemoglobin per 100 cc, and the cell-volume percentage were ascertained. Blood smears were stained with Wright's stain, and the percentages of the different types of white cells were recorded.

Post-mortem examinations were made of all animals used in the experiments; the entire alimentary tract was removed and examined for parasites and lesions. When the post-mortem examination was made soon after death, pieces of the viscera were fixed in Bouin's solution, embedded in paraffin, and submitted to the Pathological Division of the Bureau of Animal Industry for histological examination.

DATA OBTAINED

The number of infective larvae administered to the animals that survived the infestation and the number of trichostrongyles recovered post mortem from these animals are recorded in table 1. Similar data for the host animals that died as a result of the infestation are given in table 2. No nematodes were recovered from the control lamb (No. 61).

TABLE 1.—*Data on host animals with nonfatal infestations of Trichostrongylus spp.*

Animal species and No	Dates on which larvae were administered	Larvae per dose	Doses administered	Total larvae administered	Trichostrongyles recovered post mortem	
	[1] *1935*	*Number*	*Number*	*Number*	*Number*	*Percent*
Lamb 57	July 8, Aug. 12	250	36	112,750	7,004	6.212
	Aug. 20, Aug 26	500	7			
	Aug. 27, Oct 6	250	41			
	Oct. 7, Nov 10	5,000	18			
Lamb 68	Mar. 13	250	1	138,250	8	.006
	Apr. 17	1,500	1			
	Apr. 24	3,000	1			
	May 17	7,500	1			
	July 9, Aug. 12	1,000	35			
	Aug 20, Aug. 26	2,000	7			
	Aug 27, Nov 11	1,000	77			
Lamb 70	July 9, Aug 12	500	35	63,000	4	.006
	Aug. 20, Aug. 26	1,000	7			
	Aug 27, Nov 11	500	77			
Lamb 122	Nov 8	80,000	1	80,000	(2)	(2)
	1937					
Lamb 123	June 24	(3)	1	[4] 111,250	(2)	(2)
	July 27	1,100	1			
	Aug. 2	1,200	1			
	Aug. 11	2,500	1			
	Aug 17	1,450	1			
	Nov. 4	15,000	1			
	Dec. 15	90,000	1			
Lamb P2	Nov 8	100,000	1	100,000	0	.000
Total			353	605,250	7,016	
Average		1,715	58.8	100,875	1,754	[5] 1.556

[1] Administered daily for periods shown.
[2] No post-mortem examination made.
[3] Unknown.
[4] Exclusion of number administered June 24.
[5] Average for 4 lambs.

TABLE 2.—*Data on host animals with fatal infestations of Trichostrongylus spp.*

Animal species and No.	Dates on which larvae were administered	Larvae per dose	Doses administered	Total larvae administered	Trichostrongyles recovered post mortem	
	[1] *1935*	*Number*	*Number*	*Number*	*Number*	*Percent*
Lamb 58	July 8, Aug. 12	5,000	36	440,000	117,756	26.46
	Aug. 20, Aug 26	10,000	7			
	Aug. 27, Oct 3	5,000	38			
Lamb 72	Sept. 16, Oct. 12	5,000	27	135,000	63,875	47.31
	1937					
Lamb 112	Nov 19	150,000	1	150,000	83,678	55.79
Lamb 117	Oct. 22	115,000	1	115,000	80,178	69.72
Kid 127	Oct. 12	50,000	1	100,000	10,889	10.89
	Nov 8	50,000	1			
Kid 130	Sept. 25	33,000	1	78,000	52,626	67.47
	Sept. 27	45,000	1			
	Oct. 8	[2] 300,000				
Kid 131	Aug. 30, 31, Sept. 3, 10	3,000	4	196,000	29,917	15.26
	Aug. 28, Sept. 2, 4, 11, 13, 15, 16, 17, 20, 22, 23	6,000	11			
	Sept. 9, 18	12,000	2			
	Sept. 27	14,000	1			
	Oct. 6	80,000	1			
Total			133	1,214,000	438,919	
Average		9,128	19.0	173,429	62,703	41.84

[1] Administered daily for periods shown.
[2] Not included in total for reason stated in text.

The essential points brought out by the data in tables 1 and 2 may be summarized as follows: The smallest number of larvae producing death in a kid was 78,000, whereas the smallest number producing death in a lamb was 115,000. In computing the totals, the dose of 300,000 larvae administered to kid 130 on October 8, was not included since these larvae were given only 2 days before the onset of diarrhea and were therefore not considered to be responsible for the appearance of symptoms or the death of the animal. The results indicate that the kids used were somewhat more susceptible to *Trichostrongylus* infestation than the lambs. Further evidence in support of this finding was obtained from two pairs of experimental animals. Kid 130 and lamb 122 received 78,000 and 80,000 larvae, respectively, and kid 127 and lamb P2 each received 100,000 larvae, but only the kids succumbed to the infestation. The source of the infestations in the four animals was identical.

The repeated administration of small doses of larvae to the experimental animals apparently enabled them to build up some resistance and to survive the infestation. This phenomenon is illustrated by lambs 57, 68, 70, and 123. Evidence that resistance developed in the surviving animals is shown by the fact that only 1.556 percent of the larvae administered were recovered from these animals post mortem, whereas 41.84 percent were recovered from animals that died from the infestation. The average number of larvae administered to the latter group was only 1.7 times the number administered to the former.

In the case of repeated doses of larvae, if the smallest dose was 3,000 or more, the acquired resistance built up was not sufficient to protect the animal, as it developed typical symptoms of trichostrongylosis and died as a result of the infestation. This phenomenon is illustrated by data obtained for lambs 58 and 72 and kid 131, shown in table 2. These results are in general agreement with those of Gordon and Ross (*8*), who reported that daily doses of 4,000 trichostrongyle larvae were fatal to their experimental lambs.

The clinical observations made on the infested animals are recorded in table 3. As is shown in this table, the eggs first appeared in the feces of the infested animals from 17 to 21 days after infection. Host animals 112, 117, and 130, for which data on the first appearance of eggs are lacking, developed symptoms of trichostrongylosis and died before worm eggs were detected in the feces. In these three cases a positive diagnosis of trichostrongylosis could not have been made prior to post-mortem examination. That these three animals undoubtedly died of trichostrongylosis is shown by the large number of trichostrongyles recovered post mortem (table 2). Mönnig (*10, 11*) reported that the eggs of *Trichostrongylus* spp. appeared in the feces of infected sheep 21 days after infection. In the present experiments, however, eggs appeared in the feces 17 days after infection, which period is similar to that required for *Haemonchus contortus* and *Cooperia curticei*.

TABLE 3.—*Clinical observations on animals infected with Trichostrongylus spp.*

Cases, animal species, and No.	Date of first administration of larvae	Interval between first dose of larvae and appearance of eggs in feces	Maximum eggs per gram of feces	Interval between first dose of larvae and maximum number of eggs in feces	Interval between first dose of larvae and appearance of diarrhea	Date of death	Interval between first dose of larvae and death	Interval between first appearance of diarrhea and death
		Days	*Number*	*Days*	*Days*		*Days*	*Days*
Nonfatal.								
Lamb 57	July 8, 1935	21	1,800	64	-------	-------	-------	-------
Lamb 68	Mar. 13, 1935	-------	4,600	81	-------	-------	-------	-------
Lamb 70	July 9, 1935	20	2,600	49	-------	-------	-------	-------
Lamb 122	Nov. 8, 1935	19	8,200	21	-------	-------	-------	-------
Lamb 123	June 24, 1937	18	-------	-------	-------	-------	-------	-------
Lamb P2	Nov. 8, 1937	-------	5,800	36	22	-------	-------	-------
Fatal.								
Lamb 58	July 8, 1935	17	5,000	29	35	Oct 4	88	53
Lamb 72	Sept. 16, 1935	17	9,400	38	26	Nov. 16	61	35
Lamb 112	Nov. 19, 1937	-------	-------	-------	14	Dec 10	21	7
Lamb 117	Oct. 22, 1937	-------	-------	-------	13	Nov. 7	16	3
Kid 127 [1]	Oct. 12, 1937	-------	2,800	32	59	Dec 23	72	13
Kid 130 [1]	Sept. 25, 1937	-------	-------	-------	15	Oct 13	18	3
Kid 131	Aug. 28, 1937	19	[2] 54,000	63	44	Oct 30	63	19
Average	-------	18.7	5,025	45.89	28.5	-------	48.43	19.00

[1] Destroyed while in a moribund condition

[2] Not included in average, for reasons stated in text.

The maximum number of eggs per gram of feces appeared from 21 to 81 days after the first administration of larvae. The time necessary for the maximum number of eggs to appear, as well as that necessary for diarrhea to develop and for death to occur, was associated with the severity of the infestation and the rapidity with which it was acquired. These intervals were short following a single large dose or a few large doses of larvae, but were longer following small doses of larvae administered over a relatively long period. The exceptionally high maximum egg count per gram of feces for kid 131 was obtained on the day the animal died. Since the host animal had not eaten its full ration for a few days prior to death, the quantity of feces passed was considerably reduced. As a result of this decrease in the feces, the increase in number of eggs per gram of feces was out of all proportion to the actual number of eggs produced by the worms under normal conditions. Consequently, in computing the average maximum egg counts shown in table 3, the figure obtained for kid 131 was omitted.

The maximum number of eggs per gram of feces in fatal cases of trichostrongylosis varied from 1,800 to 54,000 (table 3). It is obvious that no eggs are to be found in feces of infested animals if symptoms and death occur before the worms mature, whereas a large number of eggs per gram of feces may be found if the quantity of feces expelled is less than normal. Kauzal (*9*) found that the number of eggs never exceeded 3,600 per gram of feces in the cases of trichostrongylosis that he studied. However, he quotes Gordon as reporting egg counts as high as 20,600 eggs per gram of feces. These quite different observations appear to be easily explained by the present findings.

Diarrhea, which is characteristic of trichostrongylosis, occurred 13 to 59 days after the first administration of larvae. It appeared suddenly and in most cases was continuous until the death of the host. However, there were two exceptions. Lamb P2 developed diarrhea that lasted for 3 days only, beginning on November 30; thereafter the animal showed no further symptoms. Kid 127 also developed a transient diarrhea on December 10, which disappeared after 6 days but recurred on December 21. At this time the diarrhea was profuse and continuous, and the animal became so weak that it was killed on December 23. Gordon and Ross (*8*) reported that diarrhea occurred in their experimental lambs 6 to 7 weeks after daily infection with 4,000 larvae was begun. The variation in length of the period observed in the present experiments may be accounted for by the different rate of infestation to which the animals were submitted.

In connection with diarrhea occurring in lambs affected with trichostrongylosis, Edgar (*5*) and others have mentioned the very dark green, almost black, color of the feces, from which the disease gets its common name "black scours." The diarrheic feces observed during the present investigation were not dark enough to be called black. Since little blood was ever found in the feces of the experimentally infected animals, it is probable that the black color reported by other workers may have been due to the green grass ingested by the animals or to the presence of blood in the feces caused by an accompanying infestation with *Haemonchus contortus*.

Without exception, the animals that harbored fatal infestations of *Trichostrongylus* appeared depressed, suffered from abdominal pain, and were unable to eat all the feed given them. Figure 1, *A*, shows a lamb in the last stages of trichostrongylosis; the dejected appearance, emaciated condition, and soiled hindquarters of the animal and the liquid feces on the floor of the cage (see arrow) may be noted. The emaciation of this lamb and of lamb 72, the carcass of which is shown in figure 1, *D*, was due to the inability of these animals to eat sufficient feed during the period of infestation to keep them in good nutritional condition, and to dehydration of the tissues brought about by the continuous diarrhea. In animals dying of trichostrongylosis there was no evidence of anemia, but an examination of the blood showed a marked increase in the number of leucocytes and in the percentage of polymorphonuclear neutrophiles. Andrews (*1*) also found no anemia in sheep infected with *Cooperia curticei*, and Fourie (*6*) reported a neutrophilic leucocytosis in fatal cases of haemonchosis.

Death occurred from 16 to 88 days after the first administration of larvae and from 3 to 53 days after the onset of diarrhea. Shortly before death, the animals became rapidly weaker, went into a state of collapse, and died about 2 hours later (fig. 1, *B* and *C*.)

Bennetts (*3*) reported that animals suffering from trichostrongylosis died of malnutrition resulting from loss of appetite and failure to ingest sufficient feed. In the present investigation it was found that although there was a reduction in the quantity of feed ingested by most of the animals, the severe emaciation associated with malnutrition was noted only in the animals that survived for a relatively long period. If an animal lived only a short time after infection, it reacted very much like the others, becoming weak, collapsing, and dying a few hours later. Severe emaciation occurred in the two lambs that received their infestation from day to day in a manner similar to that in which it would have been acquired from pasture. The remaining animals succumbed too quickly for a long-drawn-out process, such as starvation, to operate.

The number of worms recovered post mortem from fatal cases of trichostrongylosis was variable, as shown in table 2. This was also found to be true by Kauzal (*9*), who reported finding 35,000 to 40,000 worms commonly, and as many as 83,000 in one case. He also stated that Gordon had found one lamb that harbored 21,400 worms and another that harbored 26,200. Gordon and Ross (*8*), however, reported only 3,900 to 11,360 worms from their lambs that died of experimental trichostrongylosis.

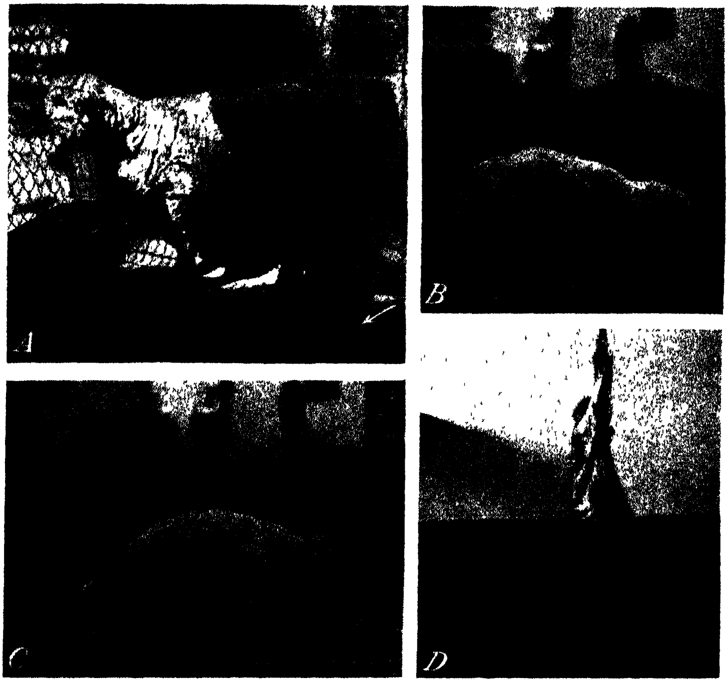

FIGURE 1.—Lamb 58: *A*, The day before it was found dead of trichostrongylosis; *B*, too weak to stand—typical of last stages of trichostrongylosis; *C*, just before death from trichostrongylosis. *D*, Carcass of lamb 72 after death from trichostrongylosis.

The following pathological changes were noted on post-mortem examination of the seven animals that died of trichostrongylosis. Serous fluid was found in the abdominal cavity, and in one case (kid 131) the mesentery was edematous. In all cases the liver was friable, and the gall bladder was distended with bile. The contents of the gall bladder of kid 127 were gelatinous and the outer surface of the distal portion of the organ was covered with a fibrinous exudate. There was diffuse enteritis throughout the small intestine in all animals, and in lamb 117 there were petechial hemorrhages in the intestinal mucosa. The pyloric region of the abomasum of kids 127 and 131 also showed some inflammation.

Edgar (*5*) stated that the outstanding feature seen on post-mortem examination was the extremely emaciated condition of the carcass. He also mentioned the absence of anemia, the presence of serous fluid in the abdomen, and the absence of lesions from the small intestine or, at most, the occurrence of minute ecchymotic areas. Boughton (*4*) reported that the mucous membrane of the entire length of the small intestine was studded with small ulcers. Mönnig (*11*) also reported the friable condition of the liver, and in addition he observed a jelly-like swelling under the throat of animals suffering from trichostrongylosis. Such a swelling was observed in kid 127 but in none of the other animals.

The histological examination of the lungs, spleen, intestine, liver, and kidneys pointed to the existence of a generalized circulatory disturbance, which had given rise to chronic passive congestion, accompanied by degenerative changes in the liver and kidneys, together with some infiltration of these areas with polymorphonuclear leucocytes. The relationship between the worm infestations, the diarrhea produced by the parasites, and the histological findings are difficult to explain on the basis of available data.

SUMMARY

In the present experiment 11 cross-bred Hampshire-Southdown lambs and 3 Saanen goat kids about 6 months old were used. The work was carried on at the Agricultural Research Center, Beltsville, Md., in 1935 and 1937.

The data show that the infestation of sheep and goats with nematodes of the genus *Trichostrongylus* usually produces a profuse, continuous diarrhea, the feces containing little or no blood. Trichostrongylosis can usually be diagnosed by the finding of the eggs of *Trichostrongylus* spp. in the feces of the infected animals. However, three experimental cases were observed in which the worms had not yet reached egg-laying maturity at the time of death of the host animals.

Under the conditions of the experiments, the smallest total number of larvae producing death in a kid was 78,000, whereas the smallest total number in a lamb was 115,000. This finding, together with the additional observation that lambs but not goats were able to survive a dose of 100,000 larvae, indicates that goats are probably more susceptible than sheep to trichostrongyle infestations.

Repeated administration of small doses of larvae to susceptible hosts enabled the hosts to build up sufficient resistance against the infestation to survive. This resistance is shown by the fact that only about 1.5 percent of the larvae established themselves and were recovered post mortem from the host animals that survived the infection, whereas nearly 42 percent were recovered from the animals that died from the infection. If the smallest dose of the series was 3,000 or more larvae, the resistance built up by the host animal was not sufficient to protect it, and death followed.

The eggs of these nematodes appeared in the feces after a minimum period of 17 days. The maximum number of eggs per gram of feces was found 21 to 81 days after the first administration of larvae and varied greatly in different animals. These observations show that the number of eggs per gram of feces in fatal cases of trichostrongylosis may vary from 0 to 54,000. It is obvious that no eggs are to be found in the feces of infested animals if symptoms and death occur before the worms mature, whereas a large number of eggs per gram of feces may be found if the quantity of feces expelled is less than normal.

Diarrhea appeared 13 to 59 days after the first infection. The variation in the length of this period, as well as that for the appearance of the maximum number of eggs in the feces and the occurrence of death, was associated with the severity of the infestation and the rapidity with which it was acquired. These periods were shorter after a single heavy infestation and longer after the administration of many small daily doses of larvae.

There was no anemia in animals dying of trichostrongylosis, but there was an increase in the number of white cells per cubic millimeter.

Although there was a reduction in the quantity of feed ingested by most of the animals, severe emaciation associated with malnutrition was noted only in those animals that survived for a relatively long period. The emaciation was apparently due not only to partial starvation but also to the dehydration of the tissues brought about by the diarrhea from which these animals suffered.

Death of the animals occurred 16 to 88 days after the first infection and 3 to 53 days after the appearance of diarrhea.

The number of worms recovered from the animals dying of trichostrongylosis ranged from 10,889 to 117,756. No extensive lesions in the intestine were found, but serous fluid in the abdomen, diffuse enteritis of the small intestine, and a friable condition of the liver were noted. A swelling was observed under the throat of one kid but not in any of the other animals.

Histological examination of the tissues taken from animals that died of trichostrongylosis indicated that death was caused by a generalized circulatory disturbance, which had given rise to chronic passive congestion accompanied by degenerative changes in the liver and kidneys. These areas were infiltrated with polymorphonuclear leucocytes.

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LIFE HISTORY OF THE NEMATODE COOPERIA CURTICEI, AND DEVELOPMENT OF RESISTANCE IN SHEEP[1]

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INTRODUCTION

Cooperia curticei, a nematode parasitic in the small intestine of sheep and goats, is of more or less cosmopolitan distribution. Curtice (*5*)[2] first reported this parasite from sheep, under the name *Strongylus ventricosus* Rud. Ransom (*28*) created the genus *Cooperia* for a group of nematodes having an anterior cephalic cuticular swelling, a lyre-shaped dorsal ray, and no gubernaculum, and selected as the type species of this genus *Strongylus curticii* Giles 1892 (=*S. ventricosus* Curtice 1890). Curtice (*5*) expressed the opinion that this nematode was of little or no importance as a cause of parasitic disease. Edgar (*9*), however, reported fatalities in goats that he believed to be the result of heavy infestations with this parasite. Ransom (*30*) and Hung (*13*) described lesions in the intestine of cattle caused by another species of *Cooperia, C. punctata;* and Schwartz (*36, 37*) and Gomez (*12*) described lesions in the intestine of a carabao caused by *C. nodulosa*. Since no detailed study of the life history of *C. curticei* appears to have been made, and since, so far as the writer has knowledge, infestations with this nematode have not been previously associated with the formation of nodules in the intestinal mucosa of the host, the following data are made available in the literature. The investigation into the life history of *C. curticei* was carried out at the Agricultural Research Center, Beltsville, Md., during 1934, 1936, and 1937.

MATERIALS AND METHODS

Specimens of *Cooperia curticei* were collected from the small intestines of sheep slaughtered at an abattoir in Washington, D. C. The female nematodes were carefully washed in physiological saline solution until they were entirely free from debris and were then cut up with a pair of fine scissors. The eggs thus liberated were cultured in a mixture of helminthologically sterile sheep feces and granular bone charcoal moistened with distilled water. This mixture was put into Petri dishes 20 cm in diameter. The lids were placed on the dishes, which were left at room temperature for about 7 days. The infective larvae that developed from the eggs were then removed from the cultures by means of the Baermann apparatus, suspended in water, and administered by mouth to a lamb by means of a funnel and rubber tube. The lamb had been raised in a cage and was relatively free from nematode parasites at the time. After infection this lamb was kept as a source of material for the further study of the parasite.

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[2] Italic numbers in parentheses refer to Literature Cited, p. 783.

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For the study of the development of the embryos and newly hatched larvae, eggs obtained from freshly passed feces suspended in water were pipetted into small watch glasses containing distilled water. The development of the embryos was followed under the low power of a compound microscope, and those in a particular stage of development were removed and studied in detail with the oil-immersion objective.

For the study of the preparasitic stages, larvae were removed from the charcoal-feces cultures at various intervals and a record made of their development at the time of observation.

In the study of the parasitic stages seven lambs were used. They were born at the Agricultural Research Center, and were less than 1 year old at the beginning of the experiment. The animals were raised in cages similar to the one in which the lamb that served as the source of infection was kept. All the lambs except three were relatively free from parasites when the experiment began. Two of these three had been fed pasture grass for several weeks prior to the experiment, and one had been on pasture during the preceding summer. Large numbers of infective larvae of *Cooperia curticei* were administered to the experimental lambs. The lambs were killed at different intervals after the administration of the larvae, and the stage of development of the parasites recorded. The presence of other nematode parasites in three of the lambs did not interfere with the present experiment since the developmental stages of *C. curticei* could be easily separated from those of the other species and from the more mature specimens of *C. curticei* that were found in these animals.

Since the life histories of nematodes closely related to *C. curticei* have been studied by Veglia (*43*), Monnig (*24*), and Threlkeld (*42*), certain details of the morphology and development observed during the present investigation that were found to be similar to those reported by the authors mentioned have been omitted from this paper.

For obtaining data on resistance of lambs to superinfection, seven animals were used. Lambs 61, 63, 65, 88, 102, and 118 were 4 to 8 months of age at the beginning of the experiment and had not previously been infected with *C. curticei.* Lamb 103, however, had been infected with this nematode during the previous summer, and was about 17 months old at the time the doses of larvae indicated in table 1 were administered. Since this lamb was used only as a source of infective material during the previous summer, no exact record of the infection was kept during that time.

LIFE HISTORY OF COOPERIA CURTICEI

PREPARASITIC STAGES

The eggs of *Cooperia curticei* (fig. 1, *A*), are provided with thin, transparent shells, with equal poles, and with sides more or less parallel. They vary from 70μ to 82μ in length and from 35μ to 41μ in width. The average length and width of 102 eggs were 76.8μ and 37.8μ, with a mean standard deviation of ±0.024 and ±0.015, respectively. When passed in the feces, the egg contains an embryo in the morula stage, having 16 to 32 cells.

When cultured at room temperature the eggs hatch in about 20 hours after being passed in the feces. The newly hatched rhabditiform larvae (fig. 1, *B*) are approximately 300μ long and grow to a length of about 500μ. The larvae enter the first lethargus about 12 hours after hatching. They become active again and cast their sheaths approximately 18 hours later, or about 30 hours after hatching.

The second-stage larvae (fig. 1, *C*) are similar to those of the first stage except that they are larger and the intestinal cells contain more

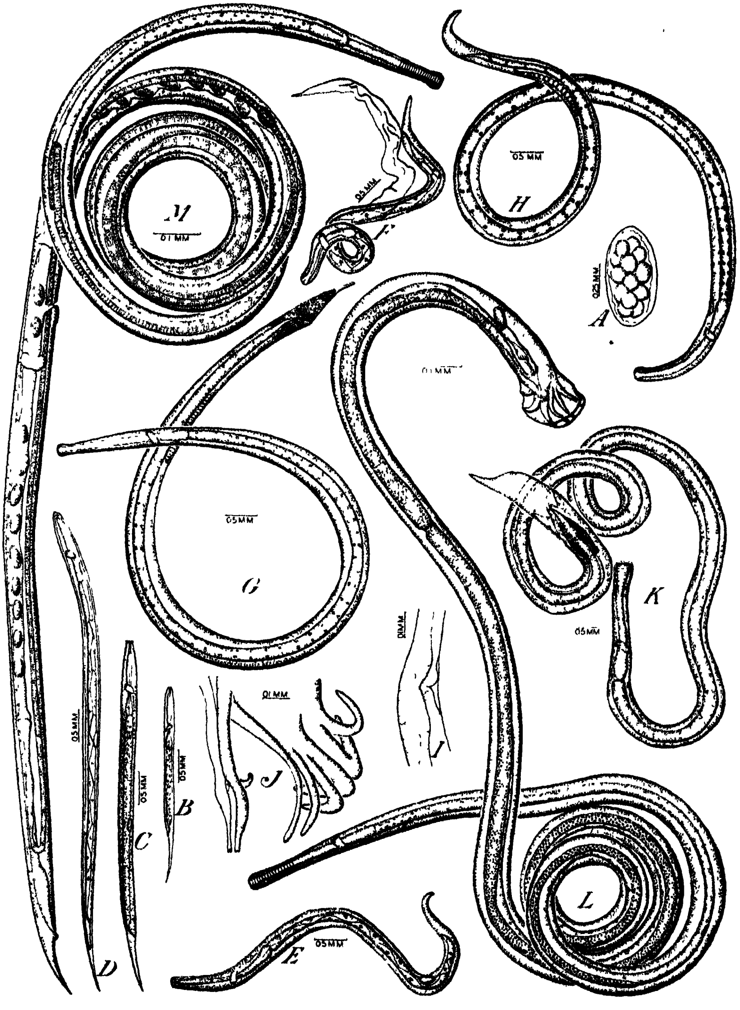

FIGURE 1.—Stages in the life history of *Cooperia curticei*. *A*, Egg; *B*, first-stage larva; *C*, second-stage larva; *D*, third-stage infective larva; *E*, third-stage parasitic larva; *F*, third-stage parasitic larva undergoing third ecdysis; *G*, fourth-stage larval male; *H*, fourth-stage larval female; *I*, formation of vagina from cells in body wall of female; *J*, bursal rays of fourth-stage larval male; *K*, larval male just after fourth ecdysis; *L*, adult male; *M*, adult female.

granules. The larvae were found to feed actively for approximately 48 hours before entering the second lethargus, which lasts about 12 hours.

About 90 hours after hatching, the larvae become active again. They have now reached the infective stage and are enclosed in the cuticle of the second stage. They measure 711μ to 860μ in length, including the sheath (fig. 1, *D*). This stage of *C. curticei* has been described previously by Dikmans and Andrews (*6*). Later, Andrews (*2*) published a figure showing that the third-stage larva of this species is characterized by having a band of fibers surrounding the buccal capsule. This observation, however, has been found to be incorrect, the bands shown in figure *B* of that paper being the transverse striations of the cuticle.

The exsheathing of the third-stage larvae was observed to take place in a very dilute alkaline solution of sodium hypochlorite (antiformin), as was previously noted by Lapage (*20*), who first reported the exsheathing of the infective larvae of other species of trichostrongyles in this solution.

PARASITIC STAGES

Two days after the experimental lambs had been infected, all the larvae recovered from the small intestines had completed the second ecdysis and appeared to be actively feeding in the lumen of the gut. They were still in the third stage and showed signs of the approaching third ecdysis (fig. 1, *E*). The lips and papillae of the fourth-stage larvae could be seen forming at the anterior end of the esophagus, which had become much thicker than that of the third-stage preparasitic larvae. The larvae appeared to increase very little in length during the parasitic part of the third stage, some of them being shorter than the infective larvae. The genital primordium had moved posteriorly, and the posterior ends of the larvae of both sexes were curved dorsally.

Three days after infection the larvae were still in the third stage, and many of them were found to be migrating into the crypts between the villi of the anterior portion of the small intestine. A large number of sections of intestinal wall were examined microscopically, and numerous larvae were seen in the crypts on the third, fourth, and fifth days after infection (fig. 2, *A*), but in no instance were they found to penetrate the mucosa.

During the fourth day after infection the larvae in the crypts passed through the third ecdysis (fig. 1, *F*) and immediately thereafter the larvae, now in the fourth stage, began to grow rapidly. Their anterior ends remained in the crypts, whereas their posterior ends protruded from between the villi into the lumen of the intestine. At this time there was great variation in the size of the larvae, those having recently passed the third ecdysis being much smaller than those somewhat older. The smallest fourth-stage larva recovered was 650μ long, but most of them were about 1.4 mm long. Sex differentiation was possible at this stage, the posterior end of the larval male being shorter and thicker than that of the larval female. The genital primordium of the female was found to be nearer the posterior end than that of the male.

By the fifth day after infection most of the larvae had returned to the lumen of the intestine. They now measured 1.6 mm in length.

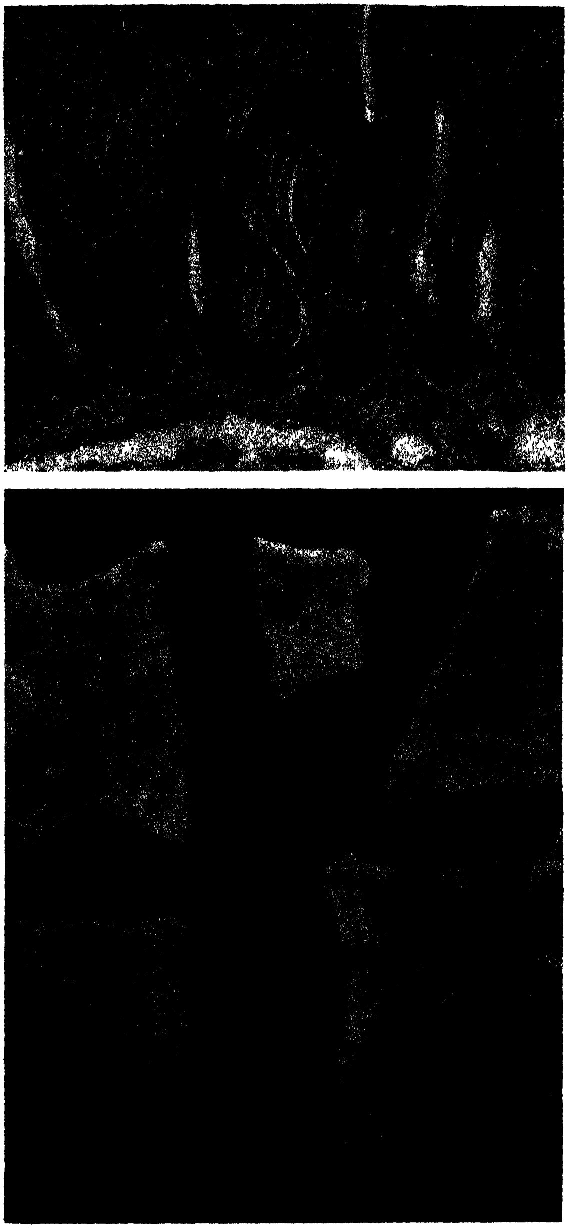

FIGURE 2.—*A*, Larvae (*see* arrows) of *Cooperia curticei* in a crypt of the intestinal mucosa of a nonresistant lamb; *B*, section of intestine showing nodules in which larvae of *C. curticei* were found.

The genital primordium of both sexes had increased in length. In the larval male (fig. 1, *G*), the genital primordium was about 260μ from the posterior end and differed from that of the female in having both germ cells at the anterior pole. In the larval female (fig. 1, *H*), the genital primordium was situated about 180μ from the posterior end and consisted of a double row of epithelial cells with a germ cell at either end. At this stage the sexes could be easily distinguished by the increased thickness and bluntness of the posterior end of the male and by the slender, pointed, posterior end of the female.

Six days after infection the larvae measured about 1.8 mm in length. In some specimens certain regions of the genital primordium had become differentiated so that the parts which form the ovaries, uterus, and ovejectors of the female, and the testis, seminal vesicle, and sperm duct of the male, could be distinguished. In the vulvar region of the female, cells could be seen in the body wall that ultimately connected with the primordium of the ovejectors to form the vagina of the adult (fig. 1, *I*). In a few specimens the lumen of the ovejectors was visible. The sperm duct of the male had not yet become connected with the cloaca. The posterior ends of the males were bilobed and the genital cone was in the process of formation. In a few specimens the spicules and bursal rays were beginning to form.

Eight days after infection the larval males were 2.4 mm long and most specimens were approaching the fourth, or final, ecdysis as shown by the loosened cuticle of the fourth stage that enclosed the larvae. The sperm duct was now connected with the cloaca, and the testis had advanced anteriorly almost half way to the base of the esophagus. Although many of the males were nearing maturity, others showed great variation in the spicules and bursa.

The rays of the bursa of a fourth-stage larval male are shown in figure 1, *J*. These rays were found to develop in a manner similar to those of *Haemonchus contortus*, *Monodontus trigonocephalus*, and *Gaigeria pachyscelis*, as reported by Veglia (*43*), Cameron (*4*), and Ortlepp (*27*), respectively. The formation of the dorsal ray by the fusion of the two adjacent dorsal branches of the longitudinal bands of the two sides in *Cooperia curticei* agrees with that reported as occurring in *G. pachyscelis* and *M. trigonocephalus*.

The larval females at this stage were about 2.9 mm long. In some specimens muscle cells could be seen in the walls of the ovejectors, which had a distinct lumen. The lumen of the vagina now connected with that of the ovejectors. The primordia of the ovaries had increased in length. The posterior ovary had already turned anteriorly after having reached the posterior limit of its development, whereas the anterior ovary had reached about half way to the base of the esophagus.

Nine days after infection, when the larvae had attained a length of about 3 mm, the fourth ecdysis occurred in the lumen of the small intestine (fig. 1, *K*). After this ecdysis the young adults matured rapidly.

Ten days after infection most of the worms had passed the fourth ecdysis. The genital system of both sexes was still immature, as shown by its size as compared with that of the adult worms, by the light-colored spicules of the males, and by the absence of eggs in the genital system of the females.

Twelve days after infection both males and females were approaching maturity, as shown by the growth of the genital system, by the darkening of the spicules of the males, and by the formation of eggs in the ovaries and uteri of the females.

Fourteen days after infection the worms had reached the size of mature individuals. The males (fig. 1, *L*) averaged about 5.3 mm and the females (fig. 1, *M*) about 6.5 mm long, respectively. The adults of this species have been described in detail by Ransom (*29*). A redescription, therefore, is unnecessary.

Fifteen days after infection the eggs were found in the feces of infected lambs.

DISCUSSION

In general, the life history of *Cooperia curticei* as reported in the present paper was found to be similar to those of the closely related trichostrongyles, namely, *Haemonchus contortus*, reported by Veglia (*43*); *Ostertagia circumcincta*, reported by Threlkeld (*42*); and *Trichostrongylus* spp., reported by Monnig (*24*). However, certain differences were observed.

The free-living phases of the life cycles of all these trichostrongyles are practically identical, since the variations reported could be attributed to the different conditions under which the larvae were cultured. However, other observed differences could not be so readily explained.

According to data reported by Monnig (*24*), the larvae of *Trichostrongylus* spp. appear to require a longer time to reach the third ecdysis than do those of *Haemonchus contortus, Ostertagia circumcincta,* and *Cooperia curticei.* This difference in the time necessary for development may explain Monnig's observation that the larvae of *Trichostrongylus* spp. increased in length during this stage of development. The larvae of *C. curticei* were found to undergo little if any increase in length during this stage, and in this respect they were similar to the larvae of *H. contortus* and *O. circumcincta.*

Unlike the larvae of *Haemonchus contortus* and *Ostertagia circumcincta,* which penetrate the mucosa of the abomasum in preparation for the third ecdysis, the larvae of *Cooperia curticei* were found to migrate superficially into the crypts of the intestinal mucosa, where they undergo the third molt. This observation was in keeping with that of Monnig, who reported a similar behavior of the larvae of *Trichostrongylus* spp. This similarity is not surprising since *Cooperia* is so closely related to *Trichostrongylus.*

According to Monnig's data, the larvae of *Trichostrongylus* spp. apparently required about 36 hours longer than the larvae of *Haemonchus contortus* and *Cooperia curticei,* and about 60 hours longer than the larvae of *Ostertagia circumcincta,* to reach the fourth ecdysis. The first-mentioned species also required 7 to 8 days longer than the period necessary for the other three nematodes to reach maturity. It has been the writer's experience, however, that under similar conditions *Trichostrongylus colubriformis* matures in about the same length of time as the other three species, the eggs being found in the feces of lambs 17 days after experimental infection.

Alicata (*1*) reported that the sex of the preparasitic larvae of the trichostrongyle *Hyostrongylus rubidus* could be ascertained by noting the relative position of the genital primordium and the fourth genital giant cell. An attempt to confirm this finding during the present study was unsuccessful. Sex differentiation in *Cooperia curticei* was not apparent until the end of the third stage, this finding being in accordance with the observations on this point made by Veglia (*43*) and Monnig (*24*) on *Haemonchus contortus* and *Trichostrongylus* spp., respectively.

DEVELOPMENT OF ACQUIRED RESISTANCE TO SUPERINFECTION WITH COOPERIA CURTICEI

EXPERIMENTAL RESULTS

During the course of the experimental infection of lambs with *Cooperia curticei*, it was noted that the number of adults recovered from the small intestine after administration of a given number of larvae by mouth varied greatly in individual host animals. It was also noted that in the host animals in which relatively few adults were found, there occurred a tissue reaction that had not previously been described as associated with infections with this parasite. This reaction was first observed at the post-mortem examination of experimentally infected lambs in 1934. It was evidenced by the presence of macroscopic nodules in the mucosa of the small intestines of the infected lambs similar to those illustrated in figure 2, *B*.

A number of these nodules were sectioned and found to contain nematode larvae. Although the larvae could not be directly identified, they were undoubtedly those of *C. curticei*, since the experimental animals had received thousands of the infective larvae of this species and had been prevented from acquiring larvae of other species. Stained sections of the nodules were submitted to the Pathological Division of the Bureau of Animal Industry for histological examination. The report on the nodules was as follows:

> The small parasitic nodules were located in the deeper structures of the intestinal mucosa, practically in contact with the basement membrane. So far as could be determined from the sections examined, the cellular elements toward the periphery of the nodules were largely lymphoid in nature, mixed with plasma cells and a few large mononuclears, whereas toward the centers, particularly of the older nodules, a larger percentage of the cells were polymorphonuclear leucocytes. Also, in some of the older nodules there was proliferation of the connective-tissue elements with a slight tendency to encapsulation of the lesions. In some of the sections there was a partial palisade formation of the connective-tissue cells at the borders of the necrotic centers and a limited number of partially degenerated giant cells were noted in a number of the nodules. Portions of the nematode larvae could be seen in the necrotic centers of a number of the older nodules.

During the examination of numerous sections of the intestine of lambs infected with *Cooperia curticei*, some larvae were found free in the crypts of the mucosa as shown in figure 2, *A*, whereas others were surrounded by various degrees of tissue reaction. The latter appeared to include all stages of nodule development, from a larva within a crypt surrounded by a few polymorphonuclear leucocytes to the formation of a complete nodule. Figure 3, *A* to *D*, inclusive, illustrates the probable formation of the nodules as postulated from these observations. According to the data obtained from a study of the slides examined, nodule formation begins with an accumulation of polymorphonuclear leucocytes about the larvae in the crypts, much as in any inflammatory process (fig. 3, *A*). The number of leucocytes increases and, together with the necrosis of tissue in the

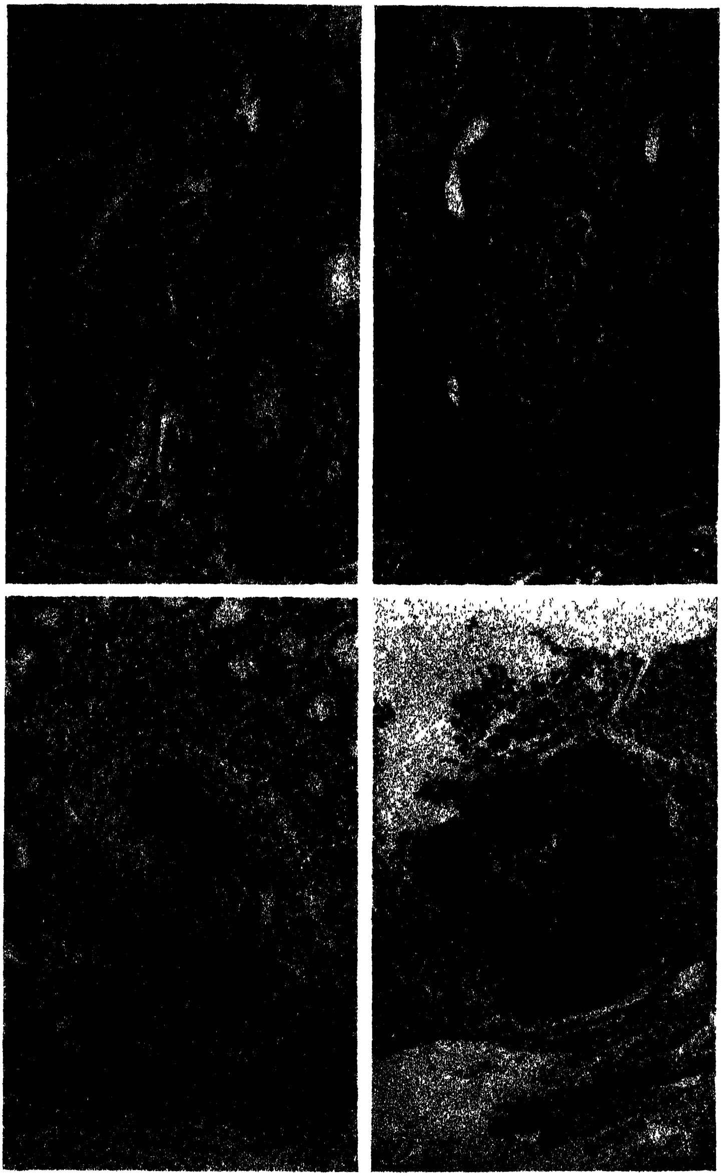

FIGURE 3.—Stages in formation of nodules containing *Cooperia curticei* larvae in the crypt of the intestinal mucosa of a resistant lamb: *A*, Early cell reaction; *B*, later cell reaction, showing increase in number of leucocytes and destruction of crypt wall; *C*, nodule formation showing beginning of necrosis of the central portion of the nodule; *D*, older nodule showing necrotic center containing a larva of *C. curticei*.

immediate vicinity of the larva, produces the condition shown in figure 3, *B*, in which the crypt is destroyed. Later, through the disintegration of the cells in the center, the lesion there becomes an amorphous, necrotic mass, and a recognizable nodule begins to form (fig. 3, *C*). Figure 3, *D*, is an illustration of an older nodule containing a large necrotic center enclosing a nematode larva. That the occurrence of these nodules was apparently related to the development of acquired resistance to superinfection is shown by the data given in table 1.

TABLE 1.—*Data on experimental infections with Cooperia curticei in seven lambs*

Lamb No.	Dates on which larvae were given	Larvae per dose	Doses given	Total larvae given	Days from first administration of larvae to death of host	Worms recovered	Worms recovered in terms of larvae given	Nodules in mucosa of intestine
		Number	*Number*	*Number*	*Number*	*Number*	*Percent*	*Number*
88	Nov. 17	10,000	1	37,500	25	7,213	19.23	0
	Nov. 22	2,000	1					
	Nov 27	10,000	1					
	Dec 1	5,000	1					
	Dec 5	3,000	1					
	Dec 7	3 000	1					
	Dec 9	4,500	1					
102[1]	Aug 3-29	5,000	27	135,000	88	25,033	18.54	0
118	Aug 27	1,000,000	1	1,000,000	5	95,960	9.60	0
63[1]	June 18-Sept 9	1,000	84	105,000	126	6,919	6.59	319
	Sept 10-Oct 21	500	42					
61[1]	June 18-Oct 21	500	126	63,000	126	8,018	12.73	1,943
65[1]	July 16-Sept 9	250	56	66,000	125	455	.007	3,273
	Sept. 10-Oct 15	500	36					
	Oct 16-Nov 18	1,000	34					
103[1]	Aug 11-Aug 14	100,000	4	400,000	16	0	000	32,000

[1] Doses were given daily to this animal for period or periods shown

The data in this table show that nodules occurred only in lambs that received daily doses of larvae over a relatively long period and in lamb 103, which had been infected during the preceding summer. Furthermore, the presence of the nodules appeared to be associated with a decrease in the percentage of worms recovered post mortem. At first glance the data for lambs 61 and 118 do not appear to bear out this statement. However, in lamb 61 the relatively high percentage of larvae recovered can be explained by the lower dose rate, which apparently resulted in the establishment of a large number of larvae before the resistance of the lamb was built up sufficiently to interfere with their development. In lamb 118, the small percentage of larvae recovered was undoubtedly due to the fact that, at the time of autopsy, most of the larvae were within the crypts of the mucosa, and, being difficult to wash out, they remained in the intestine. That this explanation is probably the correct one is borne out by the finding of numerous larvae in cross sections of the intestine of this lamb. At the time this work was done the importance of the total number of nodules was not appreciated, and the intestine of lamb 63 was placed in 10-percent formalin for preservation overnight before all the nodules were counted. This treatment rendered all but the largest nodules invisible and resulted in the small number reported from this lamb.

The fact that a smaller percentage of larvae were recovered from those lambs having nodules in the intestine indicates a definite relationship between nodule formation and the resistance of the host to infection with this parasite. The occurrence of this phenomenon in connection with the superinfection of sheep with *C. curticei* is of significance since the larvae of this nematode, so far as could be determined from sections of tissue examined, do not penetrate into the mucosa of the host. Because of this fact they have slight contact with the host's tissue, yet they are able to sensitize the intestinal mucosa. As a result, on subsequent infection the larvae are presumably immobilized in the crypts of the intestinal mucosa and are prevented from returning to the lumen where they would be able to complete their development to maturity.

The finding of worm nodules in the intestinal wall of domestic ruminants is not new. In 1876 Drechsler (*7*) described worm nodules in the wall of the small intestine of a cow that he had examined for lesions of tuberculosis. This finding was confirmed by Saake (*33*) the following year. The first report of worm nodules from the intestine of cattle in the United States was made by Smith in 1894 (*38, pp. 124–127*).

Curtice (*5*) first described nodules in the intestine of sheep in 1890, and in 1892 Giles (*11, pp. 45–49*) confirmed Curtice's finding in a report on worm nodules from sheep in India.

Since these reports, Gips (reported by Roloff and Schütz (*32*)), Janson (*14*), Ströse (*39*), Von Rátz (*31, pp. 230–232*), Scheben (*35*), Marotel (*23*), Ebhardt (*8*), Jowett (*16, 17*), Bontz and Krause (*3*), Monnig (*25*), and Liebsch (*22*) have described nodular lesions from the intestine of cattle caused by nematode larvae, and Janson (*15*), Liebe (*21*), Scheben (*35*), Joest (*15*), Theiler (*41*), Veglia (*44*), and Fourie (*10*) have described similar lesions from the intestine of sheep. Other workers have reported worm nodules from gallinaceous birds, horses, swine, monkeys, anthropoid apes, and man.

Until recently, however, the significance of the formation of nodules around nematode larvae that have invaded the tissues of the host has been obscure. Veglia (*44*) found that some of the larvae of the nodular worm of sheep (*Oesophagostomum columbianum*) failed to escape from the nodules at the end of the 5-day period, which is the usual duration of the tissue-migration phase in the life cycle of this nematode. Although Veglia associated this delay with the age of the host, he stated that purely from an anatomical point of view an encysted larva, situated too deeply in the intestinal wall or too thickly encased in an adventitious fibrous covering, might not emerge at all, or that its exit might be indefinitely delayed. Monnig (*26*) called attention to the fact that the larvae of *O. columbianum* caused a marked tissue reaction only in sheep that appeared to possess some degree of resistance to the parasite. This resistance was evidenced by the fact that the sheep which showed numerous nodules in the intestine usually had very few or no adults in the colon. Fourie (*10*) also concluded that the tissues in sheep resistant to infestation with *O. columbianum* might succeed in imprisoning the parasite.

Kerr (*18*), working with *Ancylostoma caninum* infestations in mice, showed that resistance developing in this host by previous infestation was intimately associated with great accumulations of leucocytes about the larvae, this phenomenon resulting in the retention and walling off of the latter in the tissues of the host. Kerr (*19*) found similar cellular reactions about the larvae of *Ascaris lumbricoides* in the mucosa of the ceca of guinea pigs made resistant by previous infection. Sarles and Taliaferro (*34*) and Taliaferro and Sarles (*40*) found similar cellular reactions about the larvae of *Nippostrongylus muris* in the tissues of immune rats. These authors believe that the larvae of this nematode are first immobilized by the action of a specific antibody produced in the tissues of the host as a result of previous infection. The immobilization of the larvae can be accomplished by the introduction into the host of immune serum, according to these writers. After immobilization, polymorphonuclear leucocytes accumulate about the larvae as in ordinary inflammation. These cells soon disintegrate, and with the advant of the macrophages, some of which form giant cells, and the lymphocytes, the lesion takes on the appearance of a typical foreign body reaction.

DISCUSSION

The formation of nodules in the intestinal mucosa, as has been described, is significant in the light of the preceding study of the life history. In no instance were the larvae observed to penetrate the mucosa of the intestine. They came in contact only with the epithelium of the crypts, and yet this contact was sufficiently intimate to sensitize the mucosa to the presence of larvae belonging to a later infestation and thus bring about their destruction.

Histologically, these nodules were identical with those described by early workers, and all of them were very similar to those described by Kerr, Taliaferro, and Sarles, who definitely connected the development of resistance and the occurrence of such tissue reactions with parasitic invasion.

SUMMARY

The life history of *Cooperia curticei* as determined experimentally was found to be very similar to the life history of *Trichostrongylus* spp. as reported by Monnig. When cultured at room temperature, the eggs were found to hatch about 20 hours after being passed in the feces, and the infective larvae developed about 90 hours after hatching. The infective larvae lost their sheaths soon after entering the host and migrated into the crypts of the anterior portion of the small intestine about 3 days after entering the host. However, they were never found to penetrate into the mucosa. They underwent the third ecdysis in the crypts of the mucosa on the fourth day after entering the host, grew rapidly, and returned to the lumen of the gut on about the fifth day. They continued to grow rapidly and passed through the fourth ecdysis on the ninth day after infection and matured on about the fourteenth day after infection. The eggs were found in the feces on the fifteenth day after infection.

Sex differentiation in *Cooperia curticei* was observed at the beginning of the fourth stage.

Lambs receiving daily doses of larvae over a relatively long period and one lamb that had been infected with *C. curticei* during the previous summer developed a resistance against superinfection with the nematode. This resistance was evidenced by the production of nodules about the larvae in the intestinal mucosa of the host. These nodules were found to be similar in structure to those reported by other workers.

The development of these nodules in connection with infestations with *C. curticei* was significant in that the larvae were not observed to actually penetrate the tissues of the host, yet they came into sufficiently close contact with the tissues to sensitize them to subsequent infestations.

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THE VITAMIN G CONTENT OF SOME OIL PRESS-CAKE MEALS AND RELATED PRODUCTS[1]

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INTRODUCTION

Originally the assays reported in this paper were qualitative tests of the relative amounts of vitamin G in peanut meal, cottonseed meal, soybean meal, and linseed meal by the method described by Sherwood and Halverson (*15, p. 854*).[2] Later dried brewers' yeast[3] was adopted as a standard and fed in parallel with the meal being tested in order to evaluate the sample in terms of this yeast.

REVIEW OF LITERATURE

Sometime after the inception (1930) of the work reported herein, Bourquin and Sherman (*4*) published a method for the quantitative estimation of vitamin G, which has been widely used and which has been shown to be essentially an assay of riboflavin (*2, 3, 5*). György (*8*), however, found that some factor in addition to riboflavin is necessary for growth of rats on the Bourquin-Sherman basal ration.

The outstanding difference between the Bourquin-Sherman basal ration and the one used by the present writers is that the former contains an extract of wheat as a source of vitamin B_1 whereas in the latter this vitamin was supplied in the form of an extract of rice polish, tikitiki. Morgan, Cook, and Davison (*12*) state that tikitiki is probably chiefly lacking in flavin. Bender, Ansbacher, Flanigan, and Supplee (*1*) have shown that their rice-polish concentrate contains the antidermatitis factor as well as vitamin B_1. It is reasonable, therefore, to assume that the results reported by the present writers are largely assays of riboflavin, although the influence of other members of the vitamin G complex has not been rigidly excluded and it is possible that suboptimal amounts of one or more of these uncontrolled factors may have influenced the results. The assays are reported, not as absolute values of a single vitamin, but more indefinitely as vitamin G, chiefly riboflavin.

The oil press-cake meals assayed were samples of the commercial products. Their vitamin B_1 content has been reported elsewhere (*16*).

There are few published quantitative vitamin G assays of meals from oily seeds or of the seeds themselves. Morrison (*13*) gave the vitamin G in soybeans and soybean meal a rating of 1+. Scheunert and Schieblich (*14*) stated that fresh soybeans, grown near Leipzig, contain about one-eighth as much vitamin B_2 as high-grade dried brewers' yeast. Wilgus, Norris, and Heuser (*18*) found by tests with chicks that the vitamin G in five samples of soybeans was about 3 percent of that in their standard pork liver, or about 15 percent of

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[2] Italic numbers in parentheses refer to Literature Cited, p 794.

[3] Powdered dehydrated yeast, Northwestern Yeast Co

that in dried skim milk. They also found that there was no significant difference in vitamin G in the raw seeds and the meals made from them by either the hydraulic, the expeller, or the solvent methods. Levine and Remington (*10*) found 2.4 to 3.2 Sherman units of vitamin G per gram of soybeans and 2.9 units per gram of cottonseed meal. In Daniel and Munsell's (*6*) summary of the vitamin assays of foods, soybeans are listed as containing 900 Sherman units of vitamin G per 100 g, and the same amount of cottonseed flour is stated to contain 170 units. Jukes (*9*) reported that peanut meal contains approximately 1.1 modified Sherman units per gram.

EXPERIMENTAL PROCEDURE

Albino rats, 22 to 26 days old, were given the vitamin B-complex-free basal ration previously described by Sherwood and Halverson (*15*, *16*), with distilled water, and 2 drops daily of an extract of rice polish, prepared by the method of Wells (*17*). When they had ceased to gain in weight, the basal ration was supplemented daily with a weighed amount of the product being tested or of the standard yeast, for a period of 10 weeks. The rats were housed separately in cages having raised screen bottoms.

LENGTH OF TEST PERIOD

Since the rate of gain may decrease because of the depletion of body stores of some nutritive essential not supplied in the supplement or basal ration, Bourquin and Sherman (*4*) warn against prolonging the feeding period beyond the time when the growth curves begin to flatten. The records of the present writers showed that when comparatively large amounts of the test material were fed, few of the curves flattened in 10 weeks; but when smaller amounts were fed, there was a tendency for some of the curves to break after about 7 weeks.

The coefficients of variation in gain of replicate rats getting yeast, while rather variable, usually were largest during the early part of the test period. After 4 or 5 weeks the coefficients of variation tended to reach a minimum and to remain more or less constant throughout the rest of the 10 weeks, thus indicating that the feeding period should be at least 4 weeks.

These observations, together with the consideration that in general, the longer the test period the greater the difference between comparison groups, determined the selection of the gain in 7 weeks as the criterion for judging the results.

CURVES OF REFERENCE

In order to evaluate the vitamin G in terms of yeast equivalent, in those meals which were tested before the reference yeast was adopted, a curve of reference was calculated from the results of all rats getting yeast. This curve, a straight line fitted by least squares, was found to be

$$\text{milligrams of yeast per day} = 34.14 + 1.04 \text{ (gain in 7 weeks)}$$

The standard error was 17.2, and the coefficient of correlation was 0.58.

Because of the comparatively large standard error and the low coefficient of correlation, the data were examined to see if the variation could be lessened by the introduction of corrections for other factors. First-approximation curves, drawn by the short-cut method for multiple curvilinear correlation, as described by Ezekiel (*7, ch. 16*), indicated that the time of year in which the assay was started, the initial weight of the rat, and the gain in the depletion period all influenced the gain response on a given dosage of yeast. These curves also indicated that when the above factors are considered the sex of the rat, the age at weaning, the length of the depletion period. and the weight at the end of the depletion period are of negligible importance.

The estimating curves from the completed multiple curvilinear analysis are shown in figure 1.

These curves are based upon the data from 137 rats; the details, however, are omitted for the sake of brevity. The index of multiple correlation, 0.76, was considerably higher, and the standard error, 13.6, appreciably lower than the corresponding statistics from the linear correlation. This standard error is of approximately the same order of magnitude as that found by Lindholm (*11*), although the two are not strictly comparable because he used a semilogarithmic equation for his curve of reference and expressed his error in terms of percent.

RESULTS OF ASSAYS

Two samples of dried brewer's yeast were checked against the standard yeast. One was a second lot of the same brand as the standard and the other was from a different source.[4] No significant difference was found in the vitamin G content of these three lots, which averaged 19.6 Sherman-Bourquin units per gram.

The results of the assays are shown in table 1. Three estimates of the vitamin G per gram of product are given. The first was obtained by direct comparison of the gain of the rats getting the product being assayed with that of their controls receiving yeast. This cannot be given for assays run before the standard yeast was used. The second estimate was derived by means of the curve of reference and the correction curves of figure 1. Both of these estimates are expressed in terms of milligrams of yeast required to give the same growth response as 1 g of the product. The last estimate is approximately equivalent to Sherman-Bourquin units and is based upon the amount of the product required to promote an average gain of 3 g per week. In the calculation of this value the actual gains were adjusted for season, initial weight, and gain in the depletion period by means of corrections read from curves obtained by repeating the multiple curvilinear analysis with gain in 7 weeks as the dependent variable.[5] The amount of meal required to produce a total gain of 21 g was then read from the curve, relating the corrected gain to the daily dosage of the meal.

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[4] Fleischmann's dried brewers' yeast.

[5] The standard error was 6.1 g, and the index of correlation was 0.85.

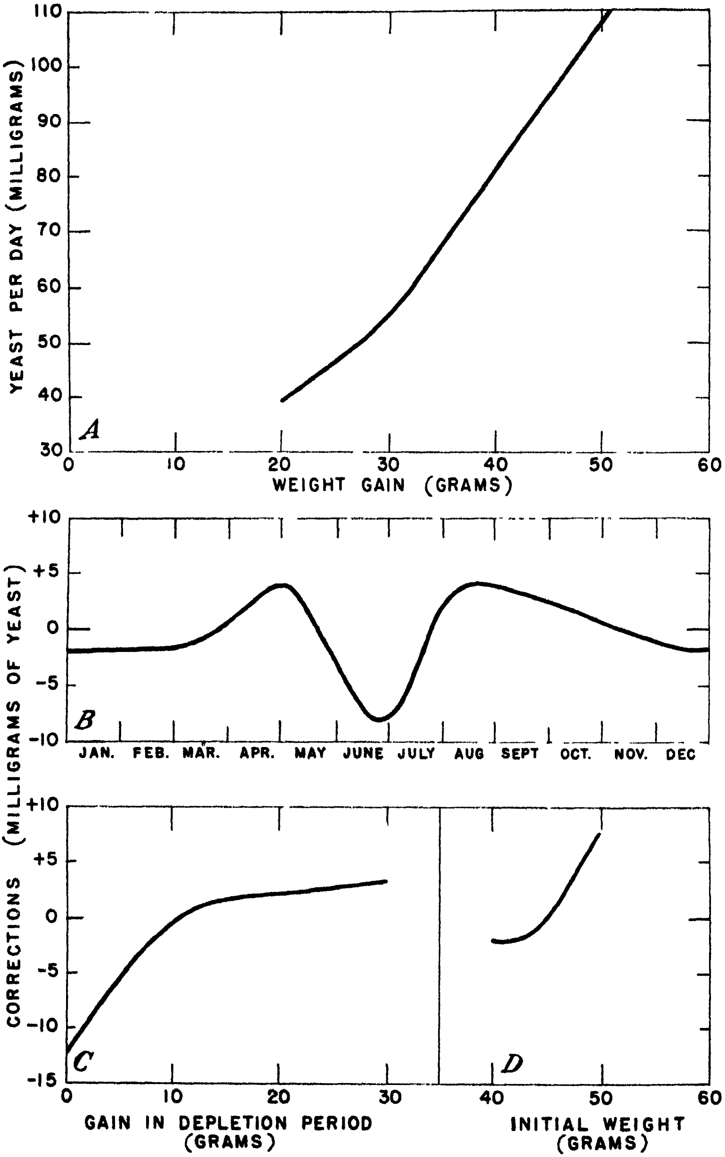

FIGURE 1.—Curves from multiple curvilinear correlation showing: *A*, Relation between the gain in weight of the rats in 7 weeks and the daily dosage of yeast per rat; *B*, correction for season (the month in which the assay was started); *C*, corrections for gain in the depletion period; *D*, corrections for the initial weight of the rats. *B*, *C*, and *D* show how variations in the respective factors affect the gain in weight produced by a given daily dose of yeast.

TABLE 1.—*Vitamin G assays of various feeding materials as estimated by 3 different methods*

Sample No	Material	Amount fed daily	Rats	Vitamin G per gram		
				As milligrams of yeast (yeast equivalent)—		Sherman-Bourquin equivalent
				By comparison with controls	From curve of reference	
		Grams	*Number*	*Milligrams*	*Milligrams*	*Units*
1	Meal from Spanish peanuts	0.3	3		145	3.7
		5	3		192	
Mean					169	
2	Meal from Spanish peanuts	4	5	102	115	2.5
		5	6		119	
		8	5	101	121	
Mean				102	118	
3	Meal from Virginia Runner peanuts	3	3		97	2.9
		5	3		132	
Mean					115	
4	Meal from Virginia Runner peanuts	4	6	150	145	2.5
		5	4	176	154	
		8	4	138	133	
Mean				155	144	
5	Cottonseed meal, 36 percent protein	3	5		112	3.3
		5	5		127	
		75	4		120	
Mean					120	
6	Cottonseed meal, 36 percent protein	3	6	147	127	3.6
		.5	6	150	126	
Mean				149	127	
7	Cottonseed meal, 36 percent protein	3	6	143	137	4.6
		.5	6	134	130	
Mean				139	134	
8	Cottonseed meal, 36 percent protein	.3	8	117	150	3.3
		.5	8	152	140	
Mean				135	145	
9	Cottonseed meal, 36 percent protein	3	8	127	160	4.3
		.5	8	120	116	
Mean				124	138	
10	Cottonseed meal, 41 percent protein	.3	9	140	163	5.5
		5	10	184	178	
Mean				162	171	
11	Cottonseed flour (Allison flour)	.3	3		170	[1] 4.1
12	Cottonseed-hull bran	.3	9	104	150	[2] 2.8
		5	10	32	82	
Mean				68	116	
13	Linseed meal	.3	8	117	150	3.3
		.5	7	124	114	
Mean				121	132	

[1] Calculated from the yeast equivalent

[2] Calculated from the mean yeast equivalent

TABLE 1.—*Vitamin G assays of various feeding materials as estimated by 3 different methods*—Continued

Sample No.	Material	Amount fed daily	Rats	Vitamin G per gram		
				As milligrams of yeast (yeast equivalent)—		Sherman-Bourquin equivalent
				By comparison with controls	From curve of reference	
		Grams	*Number*	*Milligrams*	*Milligrams*	*Units*
14	Linseed meal	0.3 .5	8 8	133 122	153 114	3.3
Mean				128	134	
15	Linseed meal	3 .5	9 9	33 103	117 110	2.5
Mean				68	114	
16	Soybean meal	5	3		162	[1] 4.1
17	Soybean meal	.3 5	8 8	77 120	140 148	3.1
Mean				99	144	
18	Soybean meal	.3 5	8 8	117 102	183 118	2.8
Mean				110	151	
19	Soybean meal	3 5	8 8	170 140	160 158	3.8
Mean				155	159	
20	Soybean meal, solvent process	3 5	8 8	73 164	190 186	5.9
Mean				119	188	
21a	Soybeans, Mammoth Yellow, stored whole, 1 year in refrigerator.	3 5	8 8	137 124	143 114	3.5
Mean				131	129	
21b	Soybeans, Mammoth Yellow, stored ground, 1 year in refrigerator	3 5	8 8	137 140	147 134	3.7
Mean				139	141	

[1] Calculated from the mean yeast equivalent.

DISCUSSION

In Sample 8 there is poor agreement between the yeast equivalents as determined by direct comparison with the controls, for the two levels of meal fed. When the yeast equivalents are calculated from the curve of reference this discrepancy is largely eliminated, but in sample 9 the difference between the results from the two dosages is enhanced by the comparison with the reference curve. In sample 15 the agreement between the yeast equivalents as determined by the two methods is poor. These are cited as examples of several other similar discrepancies in table 1. If the amount of yeast which contains 1 Sherman-Bourquin unit is calculated by dividing the mean yeast equivalent, from the curve of reference, by the Sherman-Bourquin units for each sample, the values found range from 29 mg (sample 7) to 56 mg (sample 4), whereas figure 1, *A* indicates a value of 41 mg.

So far as the immediate computations are concerned, these discrepancies are caused by differences in the slopes of the curves of response of the rats getting the yeast or the various products. Increasing the dosage level of any of these substances not only increases the riboflavin but also alters both the absolute amounts and the relative proportions of the other components of the vitamin G complex, of vitamin B_1, of protein, and of other factors which may affect the growth rate. Since no two of the products tested are identical in respect to all of these factors, it is not surprising that the slopes of the various curves of response differ.

If the Sherman-Bourquin units had been calculated from the results of that level of product which produced a gain nearest to 3 g per week, the results from the other levels being ignored, the values would have been somewhat different from those in table 1. Use was made, however, of the information obtained from feeding two or more levels by computing these units from the curve of reference as described above.

The errors (variations) of this method of assay are of two kinds: (1) Individual variation of the rats; and (2) variations in the slopes of the curves of response. An estimate of the first, the standard error of the curve of response, has been given. While the second has not been evaluated the discrepancies noted in table 1 give some idea as to its magnitude. When both types of variation are considered, there is no evidence of a significant difference in the vitamin G content of the several products. There is, however, sufficient uniformity to justify the conclusion that where comparisons are possible, the values found are in good general agreement with those given by other investigators.

If the Sherman-Bourquin values in the last column of table 1 are compared with the vitamin G content of foods as shown in Daniel and Munsell's (*6*), table 3, it is seen that peanut meal, cottonseed meal, linseed meal, soybean meal, and soybeans are good sources of vitamin G. They contain less than kidney, liver, or dried milk, which are exceptionally rich in this vitamin, but compare favorably with cheddar cheese, broccoli, beet tops, kale, and turnip greens.

SUMMARY

The term "vitamin G" is used to denote the undifferentiated complex which stimulates the growth of rats on a basal ration containing cornstarch and supplemented with an extract of rice polish but which is otherwise free of water-soluble vitamins. Riboflavin is probably the chief limiting factor in this diet.

Peanut meal, linseed meal, cottonseed meal, soybean meal, and soybeans are good sources of vitamin G, the values ranging from 2.5 to 5.9 Sherman-Bourquin units per gram. They are as rich in this factor as are cheddar cheese and the green leafy vegetables.

The optimum length of the test period was about 7 weeks The relation between gain in weight of each of the albino rats used in the experiment and their vitamin G intake (as dried brewers' yeast) was affected by the time of year in which the assay was made, and to a less extent by the initial weight of the rat and the gain in the depletion period.

A curve of reference with corrections for season, initial weight, and gain in the depletion period is given. This curve was found useful in

interpreting the results although certain limitations to its general use are recognized.

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ANATOMICAL STUDIES OF ROOT AND SHOOT PRIMORDIA IN 1-YEAR APPLE ROOTS[1]

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INTRODUCTION

Propagation of many horticultural plants is accomplished by so-called cuttings of either stem or root pieces. The degree of success attained in producing a new plant is usually dependent upon the response of the cutting in forming and developing organs that were not present when the cutting was taken from the parent plant.

In general, tree-fruit varieties are propagated either by budding or by grafting because the rooting of stem cuttings is not commercially practicable. By means of root cuttings, however, the practicability of building up populations of clons from apple (*Malus*) seedlings has been demonstrated by Luke (*6*),[2] Yerkes (*18*), and others. Shoots that have developed from root cuttings have also been readily rooted (*10, 12, 13*).

Since the report by Shaw (*9*) on the use of the nurse-root method, many workers have grown apple varieties on their own roots and thus have obtained a source of root cuttings of named varieties. Propagation by means of root cuttings, however, has not been so successful with commercial varieties as with certain seedlings (*15, 17, 19*). A summary of the results in this country up to 1930 is available (*1*). More recently, encouraging results, at least with some varieties, have been reported by Upshall (*16*).

Attention has been called to the need for an understanding of the structural features of a plant in order to interpret the responses observed in propagation by vegetative means (*8*). The anatomical study presented here on the origin and development of root and shoot primordia in the apple root may serve as an approach to subsequent experimentation on the vegetative propagation of apple roots from known varieties.

REVIEW OF LITERATURE

The comprehensive investigations dealing with the ontogeny of apical meristems by Priestley and Swingle (*8*) mark an important advance in the manner of treating the subject of vegetative propagation. The complete review given by these workers of investigations on the general problem eliminates the need of extensive comments on investigations previous to the publication of their bulletin.

Recently there has been a definite attempt on the part of investigators to determine the earlier stages in the organization of groups

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[2] Italic numbers in parentheses refer to Literature Cited, p. 802

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of cells that eventually may develop into root primordia (*2, 3, 4, 11*). Most of the investigations have been made on the origin of adventive roots on stem cuttings of various plants that were allowed to callus under the usual conditions for propagation. In rose stem cuttings, Carlson (*3*) clearly demonstrated that root primordia are initiated by small groups of cells in parenchymatous tissue in the secondary phloem. In raspberry, Sudds (*14*) reported that root primordia in various types of stem cuttings and in tip-layered canes are most frequently associated with branch and leaf traces. The origin of root primordia in pith tissue has been observed by several workers (*4, 14*). More recently, Smith (*11*) has furnished a concise report of investigations on the initiation of root primordia in stem cuttings of various plants, and summarizes the findings of the various workers. If the conclusions of the workers are accepted, it appears that root primordia may originate in practically any tissue, depending upon the plant examined. Unfortunately, the evidence has not always been supported by well-defined illustrations, and frequently conclusions have been based upon observations on relatively mature primordia.

Stoutemyer (*12*) has recently reviewed the subject of regeneration in apple and has reported on the anatomical relations of root and shoot primordia. As a result of the action of various chemicals associated with the formation of root primordia, there is opened a field which has furnished new lines of attack on this problem (*5*).

The work reported here deals with the initiation and subsequent stages in development of root and shoot primordia which, according to the writers' observations, occur as preformed meristematic "cushions" on roots of 1-year apple seedlings. These primordia may continue growth under suitable conditions after the piece of root containing them has been separated from the parent plant. They are considered as adventive under the broad interpretation of the term (*8*) in that they do not arise in a normal acropetal succession on the young root.

MATERIALS AND METHODS

Most of the material used was obtained from roots of apple seedlings grown in the greenhouse. Material from this source was fixed in October and later sectioned in paraffin by the usual histological methods; butyl alcohol was frequently used in the dehydrating series. Flemming's triple stain was used. Numerous observations were also made on roots of seedlings fixed when in a dormant condition after completing the first year's growth under field conditions.

In order to determine the relative time of emergence of root and shoot primordia, root cuttings were made from clonal material of Delicious apple and Florence crab. These root cuttings, approximately 0.2 to 0.5 cm in diameter and 7 to 8 cm long, were tied in bundles of approximately 50 each and placed in a mixture of moist sand and sawdust in the greenhouse. In addition, root cuttings of a clon of a crab apple, Vermont 323, which, in common with most other seedlings is known to propagate readily under field conditions, were used for comparison.

RESULTS

The root cuttings were examined April 11, following a period of 1 month in the sand-sawdust medium.

The results given in table 1 show the variation that may be encountered in root cuttings of different clons kept under conditions favorable for growth. Past experience has shown that larger stands may be expected from root cuttings of Florence crab than of Delicious apple. The fact that 77 percent of the Florence crab cuttings were rooted, as compared with 38 percent in the Delicious apple cuttings, is significant, especially as these figures approximate the stands generally obtained in the field for these two varieties. It seems reasonable to conclude that rooting, either in advance of the pushing of shoots or synchronous with it, is necessary for the successful propagation of root cuttings. Obviously, root cuttings that push both roots and shoots at the same time are the most readily propagated. Practically every cutting of the Vermont 323 clon, held under the same conditions but in larger numbers than the other clons, developed shoots and roots.

TABLE 1.—*Condition of root cuttings after 1 month in sand-sawdust medium*

Clonal variety	Root cuttings				
	Total examined	With roots and shoots	With roots only	With shoots only	With no roots or shoots
	Number	*Percent*	*Percent*	*Percent*	*Percent*
Delicious apple	316	31	7	22	40
Florence crab	221	20	57	3	20

ANATOMY OF THE PRIMARY ROOT

The primary root of the apple is usually characterized by a tetrarch radial protostele (pl. 1). The pericycle, at the stages shown in plate 1 and plate 2, *A*, is two layers thick, although a single-layered pericycle has been reported (*12*). The cell walls of the outer layer become somewhat thickened. The appearance of these cells does not suggest meristematic activity, and, owing to the early sloughing off of the endodermis, this outer pericyclic layer functions as a protective tissue by the time secondary growth is evident.

With the beginning of secondary growth the cells of the inner layer of the pericycle (pl. 2, *B*) begin an active tangential division, and a typical phellogen is formed by this layer. At this stage the walls of the cells in the outer layer of the pericycle are greatly thickened. This layer now resembles an endodermis; the original endodermis, however, has practically disappeared. The collapse and suberization of the cell walls of this endodermlike layer of the pericycle, accompanied by cell division in the region which was formerly the inner layer of the pericycle, marks the beginning of periderm formation (pl. 2, *C*). Fibers originate in the region capping the phloem (pl. 2, *C*) and appear to become differentiated centripetally. Because of their position, method of differentiation, and the fact that there is a rather sharp line of demarcation between them and the large parenchymatous cells of the pericyclic region lying exterior to them, they are considered part of the phloem rather than the pericycle (*12*).

Secondary roots have their origin in the pericycle opposite the protoxylem points (pl. 3, *A*). By the time the differentiation of primary tissues is complete but before secondary growth is initiated in any given transverse zone, the secondary roots have become definitely organized and have made considerable growth (pl. 3, *B*).

ADVENTIVE ROOT PRIMORDIA

The presence of adventive root primordia in most seedling apple roots can be inferred from the fact that new roots appear in regions where considerable secondary growth has been made. Histological studies demonstrate the presence of these primordia in various stages of development, as shown in plates 4, 5, and 6. Although lack of morphological uniformity makes it difficult to characterize the ontogeny of a typical initial, the illustrations indicate a progressive continuity between the very early stages and the later stages where the primordia are more completely organized.

The initiating cells are consistently found in close association with the cambial region; they are frequently associated with xylem rays (pls. 4, *A*; 6, *C*). The structure shown in plate 4, *A*, represents the earliest stage observed that can be identified with a reasonable degree of certainty. The two cells with large nuclei, at *a*, are the result of a radial division of a ray cell or a ray initial in line with the cambium. The ray cells inside have remained meristematic instead of enlarging. Additional divisions in rapid succession in the two cells with large nuclei and their progeny should result in a structure similar to the primordium shown in plate 6, *A*. The fact that these cells (pl. 4, *A*) were found in a series of sections in which numerous primordia were observed and that they lie contiguous to thick-walled cells with deeply stained contents is supporting evidence that they are initiating a primordium. Similar conditions where the apical region of the primordium is bordered by thick-walled cells have been noted to occur rather consistently (pl. 4, *B*, *C*; pl. 6, *A*).

As the young primordium develops (pls. 4, *B*, *C*; 5, *B*, *C*), there is a tendency for the apical cells to have large nuclei and to divide frequently. Later stages in development (pl. 5, *B*, *C*) show some progress toward differentiation of a definite growing point, which, however, may not be localized until the stages shown in plate 6, *A*, *B*, are reached.

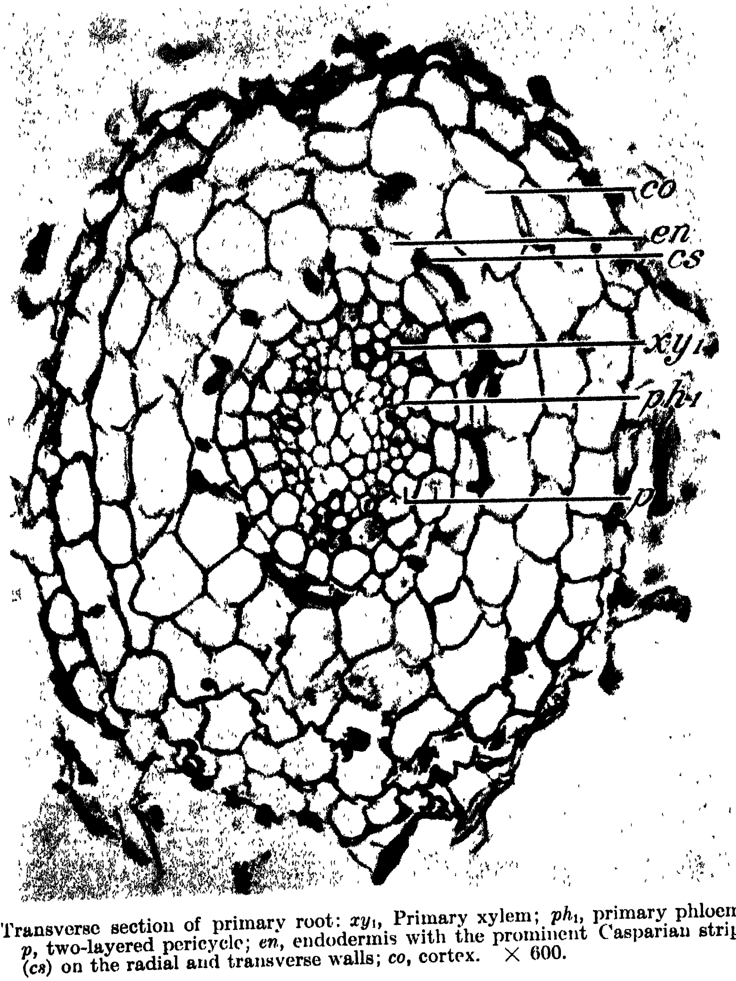
When primordia make but relatively slight forward extension, they present a "deep-seated" appearance with respect to the line of the cambium (pl. 5, *A*, *C*). There is a possibility, however, that this appearance results from partially matured ray parenchyma cells which have resumed a meristematic condition. The thin-walled meristematic condition of the cells in the xylem ray (pl. 6, *C*) is perhaps more suggestive of a resumption of meristematic properties by partially matured cells.

A comparatively few primordia were observed in which the initiating cells seemed to be several cells distant from the cambium (pl. 6, *D*), but it is difficult to determine accurately the origin of primordia that have reached this stage of development.

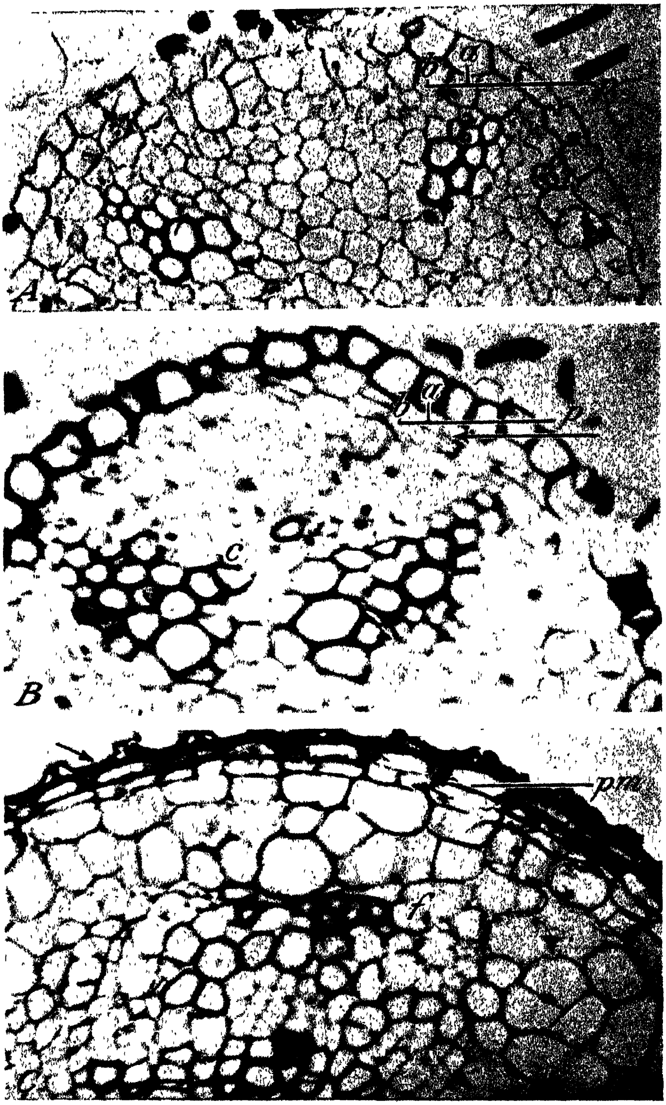
ADVENTIVE SHOOT PRIMORDIA

In contrast to the number of investigations on the origin of root primordia, there has been relatively little work on the origin of shoot primordia. Investigations to date have been summarized (*8*, *12*), but it is difficult to draw general conclusions.

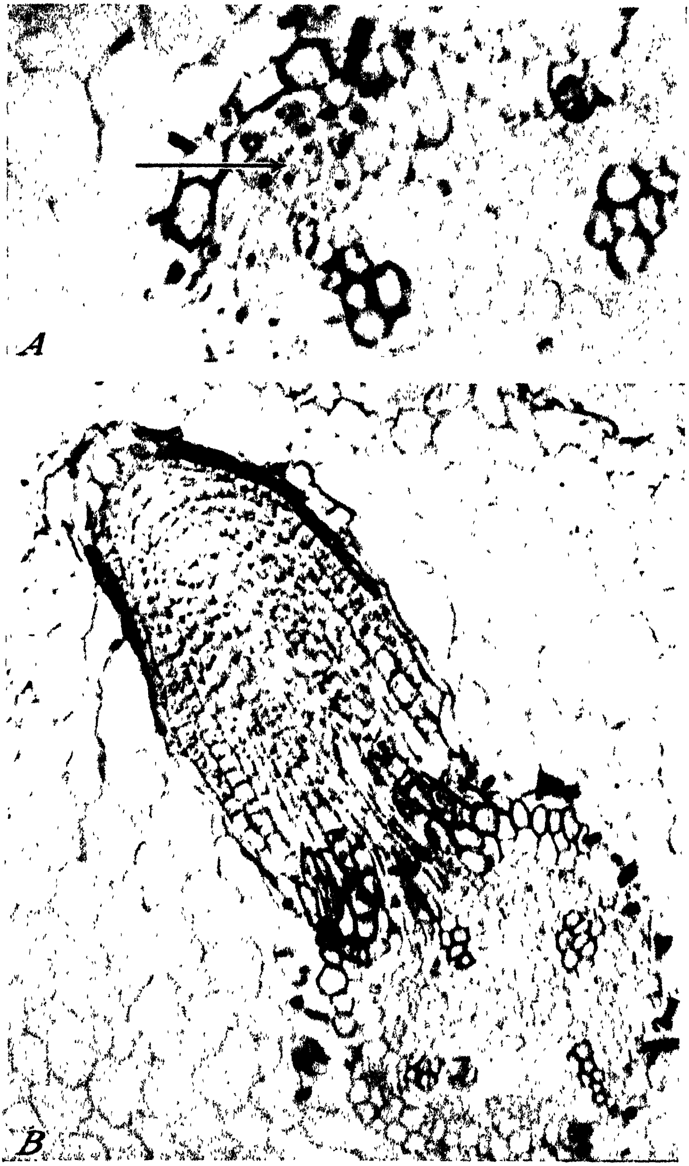
The illustrations were selected to show progressive stages in the development of shoot primordia from a small group of initiating cells to a completely organized structure (pls. 7–11). In the material used



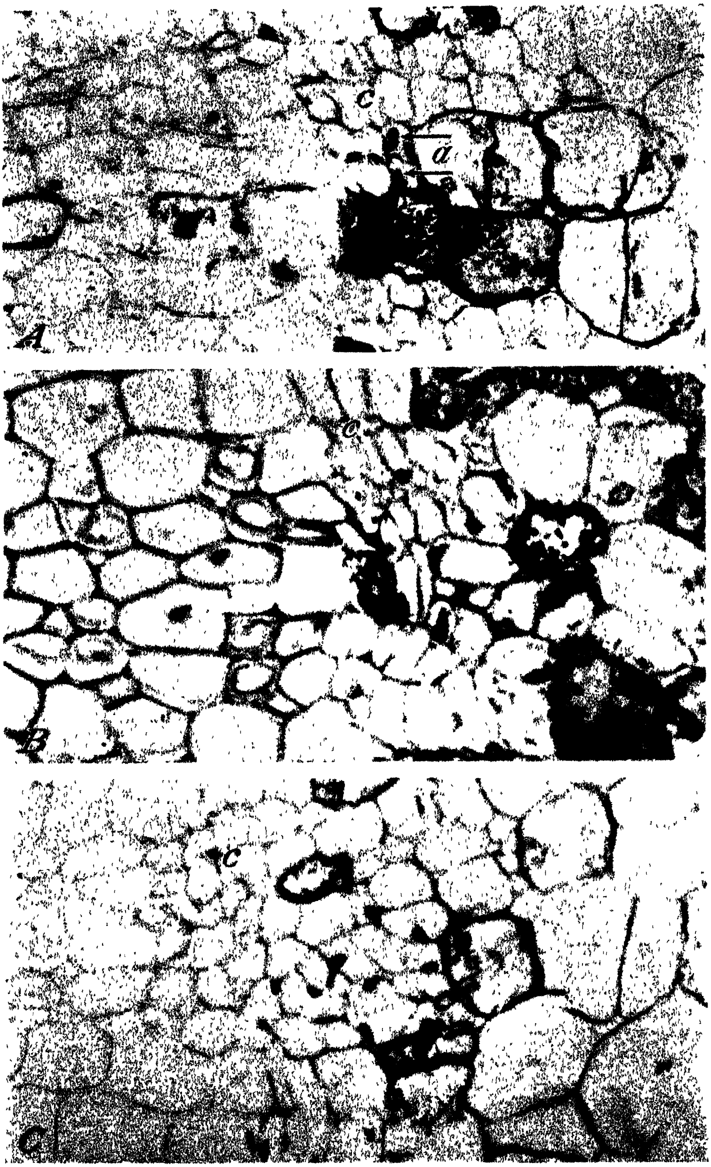
Transverse section of primary root: xy_1, Primary xylem; ph_1, primary phloem; *p*, two-layered pericycle; *en*, endodermis with the prominent Casparian strips (*cs*) on the radial and transverse walls; *co*, cortex. × 600.



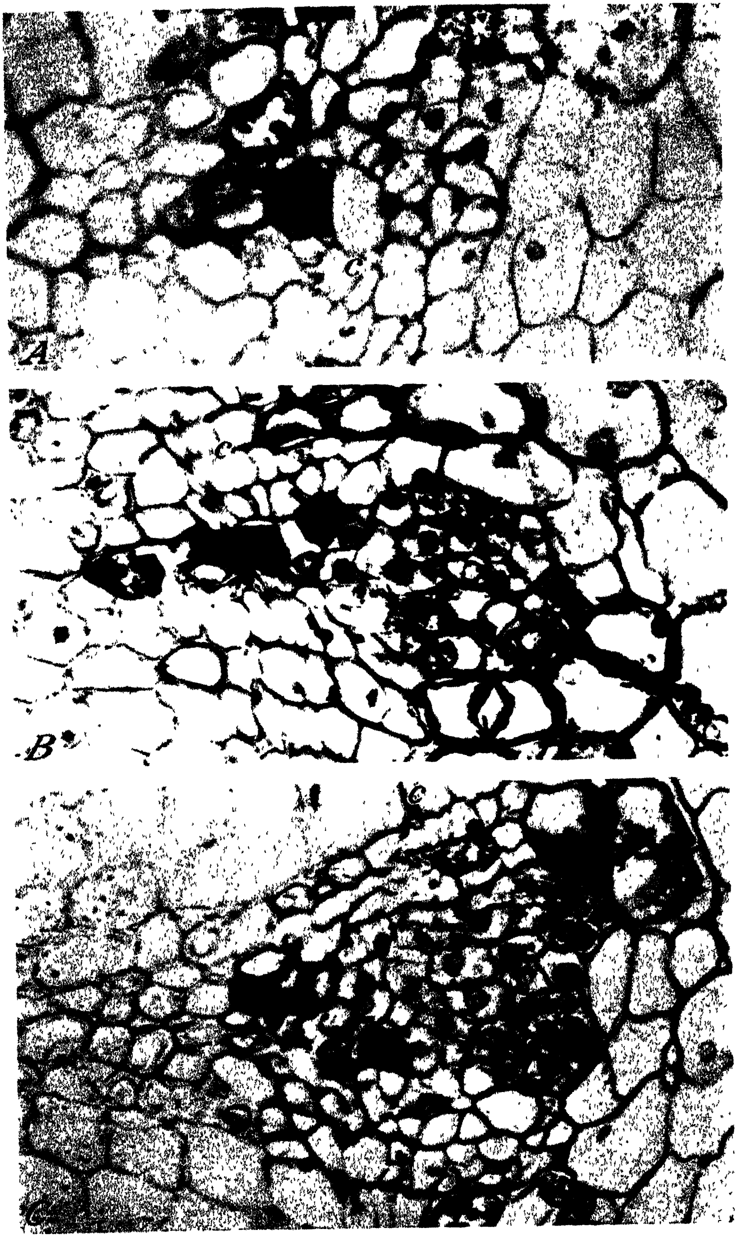
A, A late stage in primary growth. The endodermis has practically disappeared. The cells of the outer layer of the pericycle (*p*), at *a*, have comparatively thick walls; the inner layer of the pericycle (*p*) is shown at *b*. × 600. *B*, From the derivatives of the cambium (*c*) xylem and phloem elements are being differentiated. The cell walls of the outer layer of the pericycle (*p*), at *a*, are greatly thickened: this layer now resembles an endodermis. The inner layer of the pericycle (*p*), at *b*, shows numerous tangential division (indicated by arrow) and is beginning to develop a phellogen. × 600. *C*, The collapsed cells (indicated by arrow) of the original outer layer of the pericycle, together with the activity of the phellogen, mark the beginning of periderm formation (*pm*). Fibers (*f*) are present, capping the phloem elements. × 425.



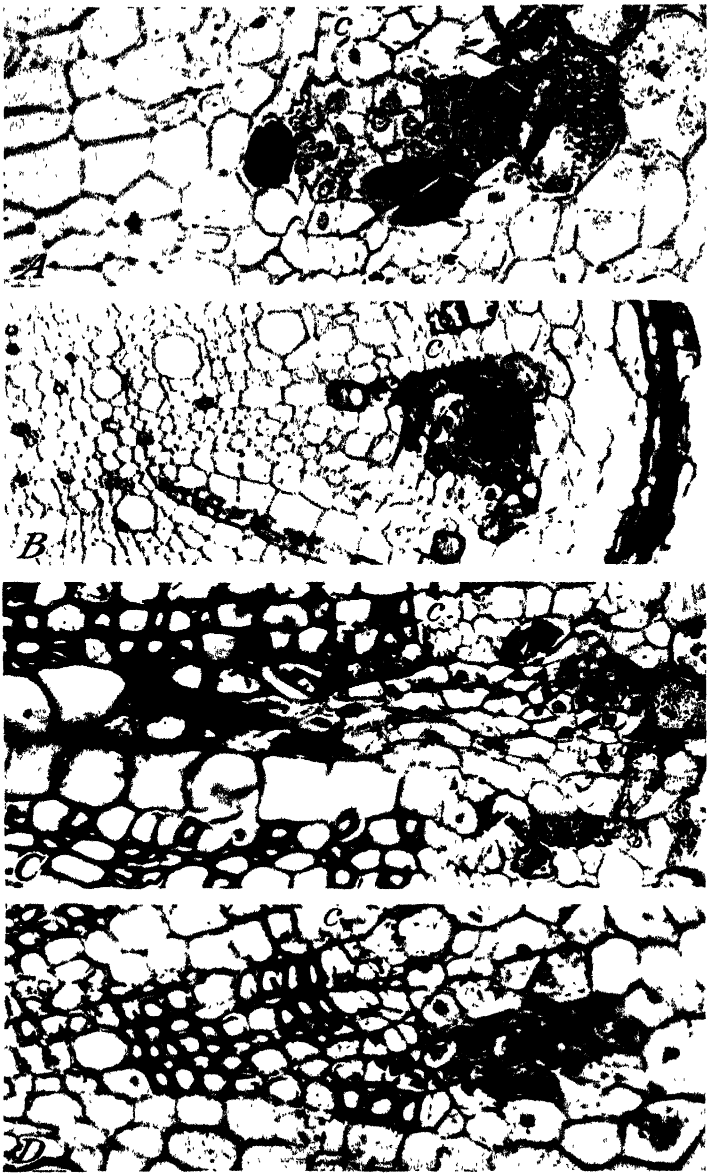
A, The initiation of a lateral root from the inner layer of the pericycle during primary growth, × 600; *B*, the lateral root is definitely organized before secondary growth in the parent axis, × 250.



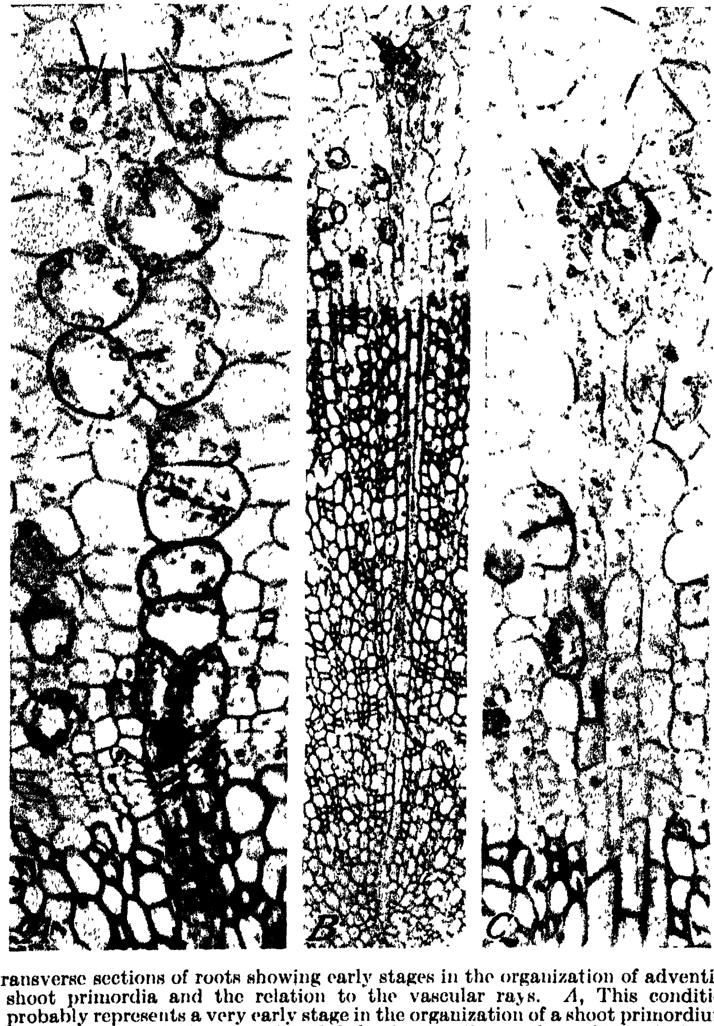
Transverse sections of apple roots showing origin of adventive root primordia in proximity to the cambial region (c), × 575: *A*, An early stage of a primordium, indicated by the two cells with large nuclei (indicated by arrows) at *a*, which have resulted from a radial division of a ray cell in a line with the cambium; *B*, *C*, early stages in the formation of primordia, whose apexes are partially bordered by thick-walled cells containing deeply stained contents.



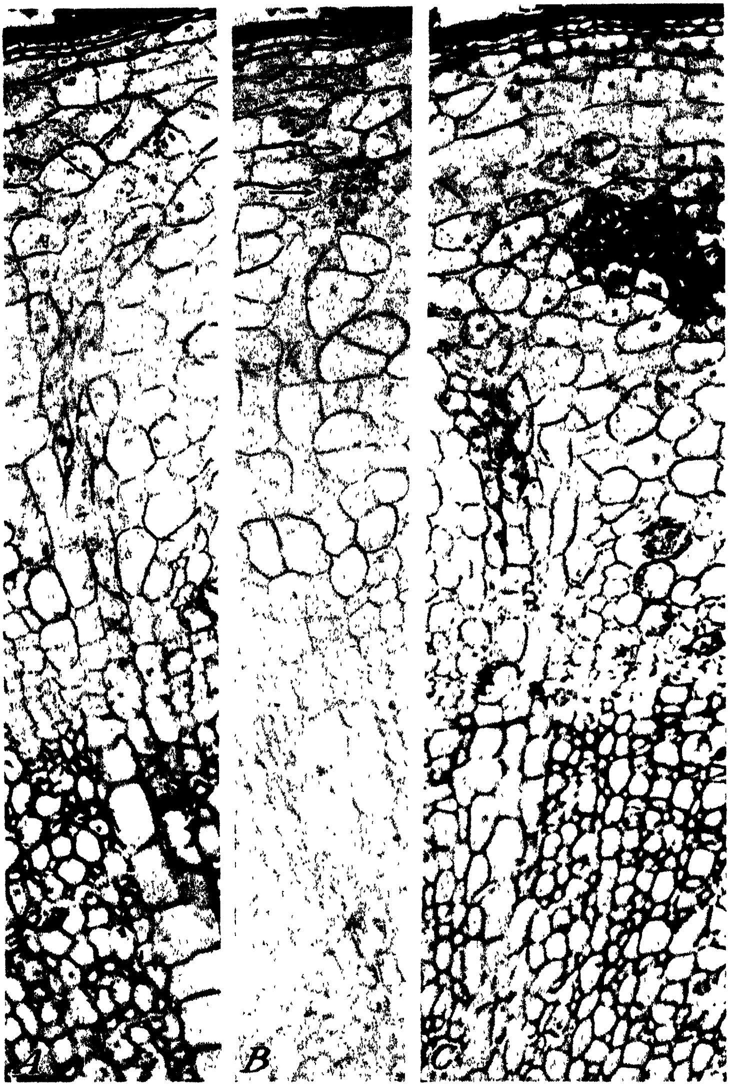
Transverse sections of roots showing later stages of primordia. *A*, × 550. *B*, *C*, The apical areas which contain cells with large nuclei are becoming organized into growing points. Present line of the cambium is at *c*. *B*, × 575; *C*, × 450.



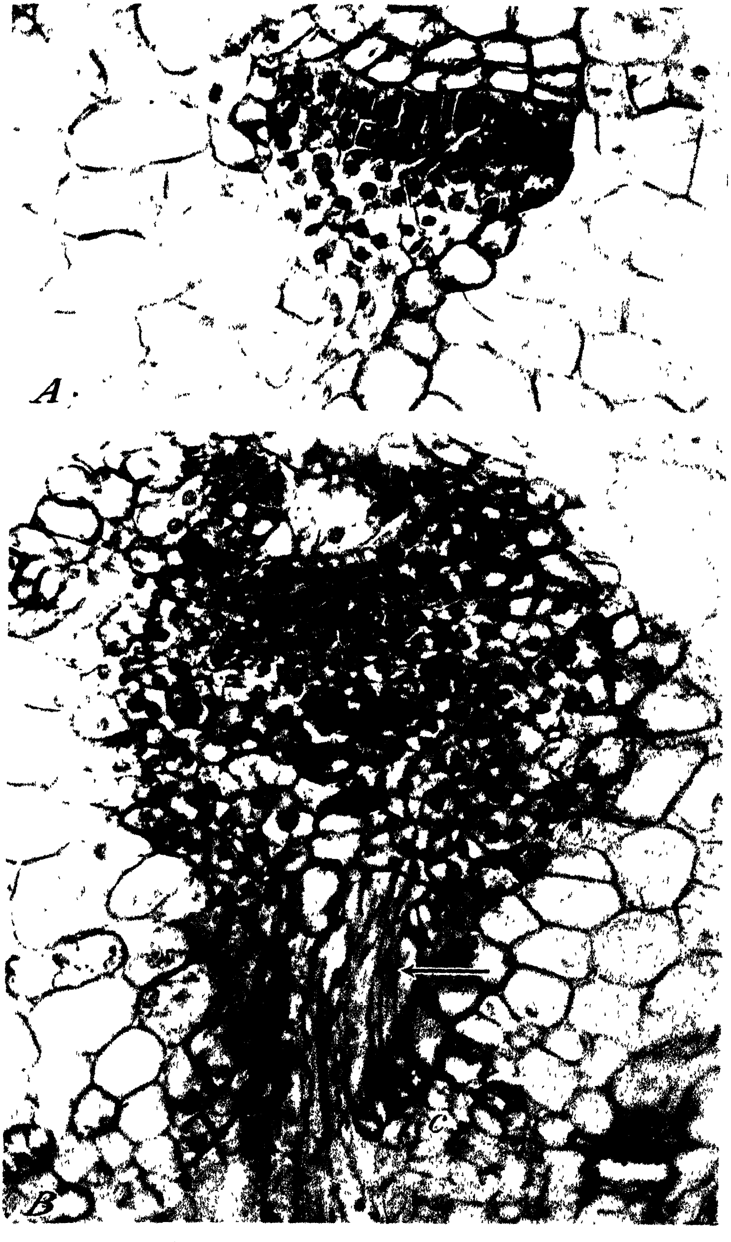
Transverse sections of roots showing adventive primordia in various stages: *A*, Organization of the growing point, × 575; *B*, a "mature" primordium, × 250; *C*, ray parenchyma cells in xylem becoming meristematic, × 325; *D*, a type of primordium, observed infrequently, in which the initiating cells may not have been intimately associated with the cambium (*c*), × 425.



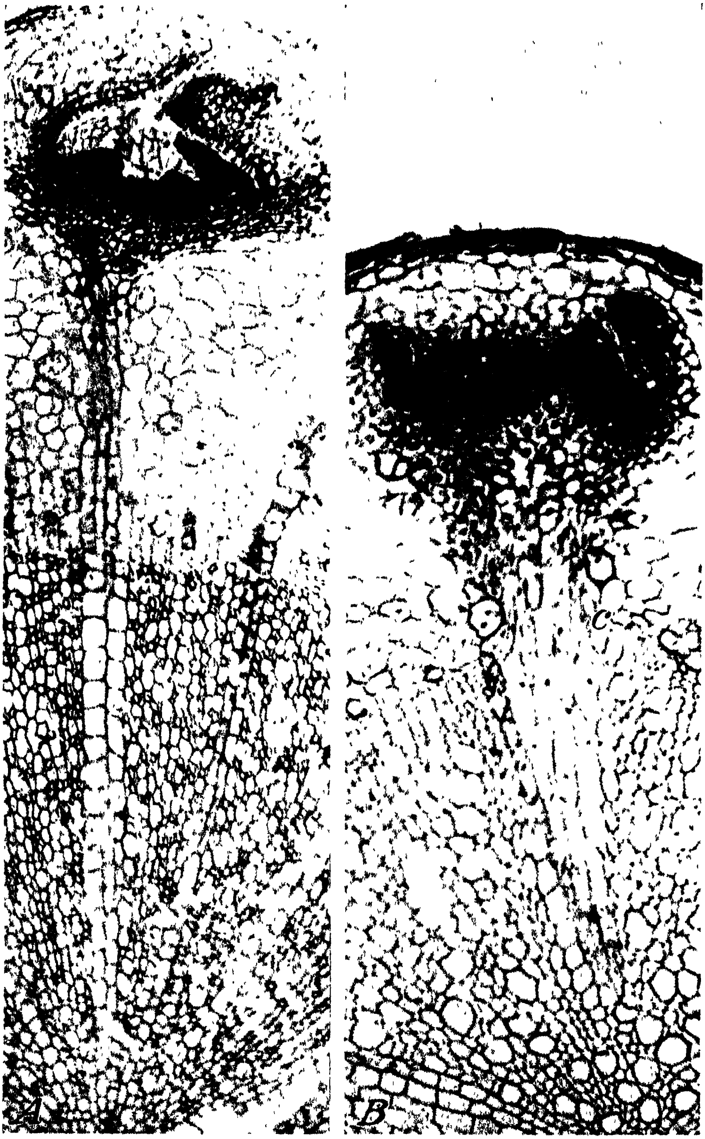
Transverse sections of roots showing early stages in the organization of adventive shoot primordia and the relation to the vascular rays. *A*, This condition probably represents a very early stage in the organization of a shoot primordium. At the distal end of a row of well-defined ray cells are three thin-walled cells (indicated by arrows) which contain small but deeply stained nuclei. × 325. *B*, A ray in which meristematic activity is especially pronounced at the apex in the phloem region. × 100. *C*, Phloem area of ray in *B* enlarged. × 250.



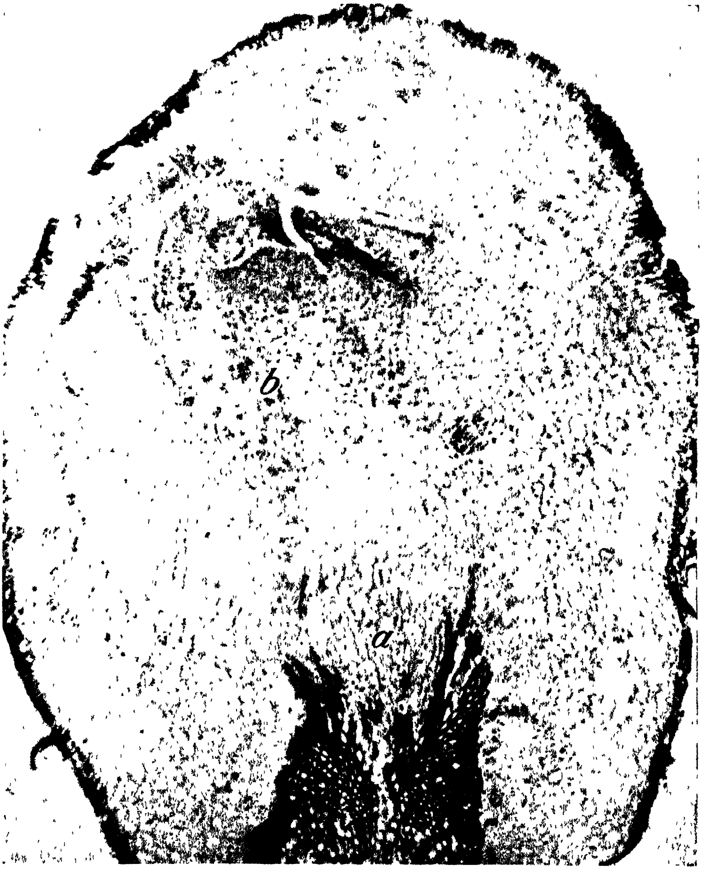
Transverse sections of root, × 175. *A*, Meristematic activity of ray cells. *B*, Serial section cut approximately 50μ distant from the section shown in *A*. The group of cells (indicated by arrow) with prominent nuclei is at the apex of the ray cells shown in *A*. *C*, A general relationship of the dense aggregation of cells at the apex of the primordium to the vascular ray is indicated.



Transverse sections of roots showing shoot primordia: *A*, A single-layered dermatogen is being differentiated, × 450; *B*, foliar initials are forming and, at the base of the primordium, the three elongated cells (indicated by arrow) will presumably function as cambium (*c*) to effect a vascular union between the primordium and the xylem of the parent tissue, × 350.



Transverse sections of roots showing shoot primordia. *A*, The distal (apical) region of the primordium is completely organized, but there is comparatively little change in the parts of the ray behind this apical region. × 100. *B*, Efficient "connections" are established between the apical region and the xylem of the parent root. Elongated meristematic cells at the proximal part of the primordium are in line with the cambium (*c*) of the parent tissue. × 150.



Transverse section of root showing an adventive shoot primordium as it is about to emerge from the parent root. The original cambial cylinder surrounding the xylem is broken, and growth to the inside has formed a large gap at *a*. The present line of the cambium enclosing this gap is continuous with the line of the procambium, which is differentiating protoxylem at the point indicated at *b*. × 75.

very few early stages were found, and it would be desirable to make additional studies on these early stages.

Shoot primordia were almost invariably found in association with vascular rays. In the apple root these rays are prominent and can be readily recognized as a continuous tissue extending from the protoxylem to a region near the periphery of the root. Meristematic activity in several ray cells near the periphery, accompanied generally by increased activity centripetally in the ray cells, appears to be the mechanism favorable for the initiation of the primordia.

In plate 7, *A*, the ray cells in the phloem are fairly conspicuous. This of course is normal, but in this case the three cells that mark the apex appear to be more meristematic than usual. The nuclei in these three cells are more deeply stained, although they are somewhat masked by the amyloid cell contents; the cell walls are very thin and in some portions are indistinguishable. This condition suggests a resumption of meristematic activity by these cells, and therefore this structure is tentatively identified as a very early stage of a shoot primordium.

The group of cells shown in plate 7, *B*, *C*, can be definitely identified as an initial stage. The nuclei at the apical region are very small but have taken an intense stain; the cell walls are barely discernible. Meristematic activity is also evident behind this region but decreases progressively toward the cambial region.

The aggregation of cells, with small, deeply stained nuclei, near the periphery in plate 8, *B*, actually marks the apex of the primordium shown in plate 8, *A*. The relationship of these areas can readily be visualized by figuratively superimposing this group of cells (pl. 8, *B*) upon a corresponding area of the ray in plate 8, *A*.

With the increase in size, it obviously becomes more difficult to secure orientation to include representative cross sections. Thus the relation of the dense, meristematic apical region of the primordium to the meristematic ray cells behind it (pl. 8, *C*) appears somewhat indefinite. Later stages in the development of primordia are shown in plates 8, *C;* 9, 10, and 11. From the unorganized mass of cells (pl. 8, *C*), the apical end of the primordium has differentiated a dermatogen (pl. 9, *A*), and foliar initials are noted in the stage shown in plate 9, *B*. The growing point has become organized in a manner similar to that described for syringa (*8*). The region of the growing point may be well organized in the absence of coordinated activity of the cells in the regions behind it (pl. 10, *A*). On the other hand, a primordium whose apical region is in a similar stage of development but which also shows complete organization as a unit with the parent tissue is shown in plate 10, *B*. Presumably this latter type of primordium, because of the completeness of its connections with the vascular tissue of the parent axis, is well adapted for survival upon emergence from the parent tissue. This anatomical consideration may explain why in shoots that emerge from root cuttings there is mortality of some, and variation in growth of others.

Ultimately, connections between the primordium and the vascular tissue of the parent root are established by differentiation of meristematic cells. Plates 9, *B*, and 10, *B*, show tagential and median longitudinal sections, respectively, of the same primordium. The elongated meristematic cells at the base of the primordium are oriented at right angles to the cambium of the parent tissue and presumably will establish a cambium cylinder at the proximal part of the primordium. By the time the stage of development shown in plate 11 is reached, a considerable amount of xylem has been laid down and the line of the cambium is continuous with the procambium from which protoxylem has developed at *b*.

It should be noted that the primordium shown in plate 11 was formed toward the end of the growing season and remained as a semidormant cushion during the dormant period of the plant. Upon resumption of suitable growing conditions in the spring, preformed primordia of this type develop rapidly. Numerous leaf primordia are formed before the shoot emerges from the cortex.

DISCUSSION AND CONCLUSIONS

In apple roots, so-called preformed root and shoot primordia are developed on the current season's growth. There is considerable variation in the frequency of occurrence of these primordia, not only in seedlings of different parentage but among different clons. The success attained in propagating root cuttings of clons is largely dependent upon the presence of these preformed primordia in the root cutting when it is taken from the parent plant. The presence of shoot primordia in particular can be frequently detected with the unaided eye by the end of the growing season. These primordia, occurring as cushions at numerous points on the roots, are generally found in abundance on seedlings of crab apples, including named varieties such as Florence, and on seedlings of commercial varieties. Observations over a number of years have led the writers to the conclusion that in general cuttings from roots that exhibit macroscopically a large number of primordia cushions are propagated most successfully.

A correlation of the anatomical studies reported here with the investigations of most other workers is difficult because of lack of similarity in the material used. Priestley and Swingle (*8*) present detailed anatomical analyses on a number of plants. Investigations in general have dealt with the production of root primordia on stem cuttings of herbaceous and woody dicotyledons. With few exceptions (*2*), the workers previous to Priestley and Swingle were concerned with noting the presence of primordia rather than with detailed anatomical studies of their origin. Unfortunately, conclusions as to the origin of primordia have frequently been made as a result of observations on mature structures. The present discussion deals primarily with initial stages of the primordia and their growth and development in root tissues only.

It should be noted that the illustrations of primordia are taken chiefly from roots that have made approximately one season's growth and represent the conditions usually found in root pieces approximately 2 to 5 mm in diameter. A few structures that could not be definitely identified were observed.

A feature not commented upon in the remarks on the illustrations is the fact that in the regions where primordia were found in greatest numbers phloem fibers were generally lacking. Additional observations are necessary before the significance of this observation can be evaluated (*7*).

The studies on the anatomy of the young root, including primary and some secondary growth and the locus of origin of the primordia, illustrate the suggestion of Priestley and Swingle (*8*), viz, that the environment in which a cell or group of cells is located is an important factor in determining the future course of development. Owing possibly to the presence of the prominent Casparian strips, the endodermal cells do not exhibit much evidence of divisions; they are sloughed off before primary growth has ceased. In turn, the walls of the outer layer of the two-layered pericycle become greatly thickened, and this layer functions as protective tissue; the inner layer of the pericycle eventually functions as a phellogen. All adventitious primordia originate after considerable secondary growth has taken place and therefore are "endopericyclic" and, in the sense of the term as used by Priestley and Swingle, would be considered endogenous.

That adventitious root primordia were formed comparatively late in the growing season is indicated by the relative stages of organization of the more mature root and shoot primordia. Shoot primordia were usually more completely developed than root primordia. This fact may explain why many root cuttings, when planted, produce numerous shoots that may make considerable growth before the adventive roots have developed proportionately. As a result of this paucity of roots, a high mortality may result (*17*, *19*). However, with the root cuttings held in the greenhouse under conditions favorable for growth, the fact that cuttings of the Florence crab variety in general exhibited root growth in advance of shoots shows that variations may be expected not only between different clons but also between individuals of the same clon.

The evidence obtained from a study of very early stages of root primordia indicates a close association between the initiating cells and the cells of the vascular cambium, or at least their close derivatives. There is no consistent relation of the primordia to the vascular rays. The small group of cells at the apex of the young primordium almost invariably is contiguous to, and apparently delimited by, groups of parenchymatous cells possessing comparatively thick walls and deep-staining cell contents. Possibly there is here a relationship similar to that postulated for the function of the endodermis (*8*).

Because of the close association of the early stages of the root primordium with the cambium, continuity with the mature vascular tissues of the parent axis is readily established. Those root primordia that originate in a ray probably have an advantage in subsequent growth, because the parenchymatous ray cells have the potential meristematic properties which would result in differentiation of cells capable of effecting an optimum anatomical union with the xylem tissues of the parent axis.

The ontogeny of adventive shoot primordia has been discussed previously in some detail in connection with the individual illustrations. The literature dealing with the origin of adventive shoot primordia is decidedly meager and is concerned chiefly with herbaceous plants (*8*, *12*). In this paper emphasis is placed on the probable role of the vascular rays in the formation of shoot primordia. The genetic connection and homogeneity of the tissue extending from near the protoxylem points to a region near the periphery of the mother tissue apparently provide an environment favorable for initiation of shoot primordia. Although the ray cells may differ in appearance because of their cellular environment, they are a relatively homogeneous unit in function and in structure. It is therefore not surprising that these ray cells act as a unit in the formation of the primordium. It should be noted, however, that Stoutemyer (*12*) reports that bud primordia do not arise at any definite points with respect to the vascular rays.

Manifestations of differentiation in the ray cells first become apparent in the periphery of the parent tissue, as has been discussed. The relative activity may vary in the regions destined to be the proximal and distal ends of the primordium. As a result, the vascular connections established with the parent tissue may vary greatly between different primordia. It is permissible to postulate that the establishment of adequate vascular connections presages successful growth of the young shoot.

SUMMARY

The origin and development of adventive root and shoot primordia in 1-year apple roots have been studied in order to obtain an explanation, based on anatomy, of the vegetative response.

The anatomy of the primary root is described to show the origin of normal lateral roots and the differentiation of secondary tissues.

Adventive root and shoot primordia have their origin in tissues that have made considerable secondary growth.

Adventive root primordia originate in the region of the vascular cambium, either in ray parenchyma cells in a line with the cambium or in near derivatives of cambial cells not associated with rays.

Shoot primordia are organized as a result of meristematic activity of ray parenchyma cells. Activity in a small group of ray cells near the periphery is accompanied by divisions in the posterior regions of the ray frequently extending into the xylem. The conception that the ray cells exist as a homogeneous genetic unit through the xylem and phloem regions is advanced to explain the manner in which the shoot primordium is organized.

In the material observed, shoot primordia were generally more completely organized than root primordia. This condition probably accounts for the relatively quick emergence of shoots in plantings of certain clons and the resulting mortality due to lack of roots sufficient to support the top growth. Variations in the manner in which the vascular connections are established between the shoot primordium and the parent tissue probably explain variations in the vegetative response of shoots growing from individual root cuttings.

The vegetative response of root cuttings of several clons indicates that successful propagation is largely dependent upon the ability of the cutting to push roots either in advance of shoots or concurrently with them.

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BROWN NECROSIS, A DISCOLORATION ASSOCIATED WITH RUST INFECTION IN CERTAIN RUST-RESISTANT WHEATS[1]

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INTRODUCTION

In recent years many conflicting statements concerning the nature and symptoms of the black chaff disease of wheat (*Triticum aestivum* L., syn. *T. vulgare* Vill.), usually attributed to the organism *Bacterium translucens* var. *undulosum* Smith, Jones, and Reddy, have appeared in the literature on cereal diseases. Several investigators have reported that the rust-resistant varieties Hope and H–44 and many of their derivatives are very susceptible to attack by this organism. Because of the prevalence of a so-called black chaff in the rust-resistant hybrid segregates from these wheats, some concern has been expressed over the possible results should such wheats become widely grown. For this reason many strains of wheat descended from Hope and H–44 that appear to be peculiarly susceptible to black chaff are discarded every year from various wheat-breeding nurseries. The importance of knowing the true cause and probable future economic importance of the disease is, therefore, quite obvious.

This paper presents experimental data, together with certain evidence from publications of other investigators, which would seem to demonstrate that much of the so-called black chaff reported in Hope and H–44 and their rust-resistant derivatives is merely a manifestation of resistance to stem rust. In this paper, the term "brown necrosis" is applied to the melanistic reaction or discoloration that develops in certain varieties in the presence of stem rust.

REVIEW OF LITERATURE ON MELANISM IN RUST-RESISTANT WHEATS

In previous publications[3] (*10*)[4] the writer drew attention to the "association," in selections from a Yaroslav emmer × Marquis cross, of susceptibility to "black chaff" and "anthracnose" with a type of resistance to stem rust which was derived from the Yaroslav emmer parent. It was pointed out[5] that the association of resistance to

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stem rust with susceptibility to black chaff was not complete. The belief also was expressed (*10*) that some of the pigmented areas in the vicinity of the nodes of the emmer derivatives were the result of infection by stem rust. Hart and Zaleski (*7*) have since presented evidence which supports this opinion and have also stated that some of the pigmented areas surrounding the points of infection by stem rust resembled the lesions produced by *Bacterium translucens* var. *undulosum*.

Waldron (*12*) reported an "antagonistic relationship" between black chaff and stem rust in hybrids of Hope wheat. Hart and Zaleski (*7*), however, found no antagonistic relationship between stem rust and the true black chaff disease caused by *Bacterium translucens* var. *undulosum*.

Ausemus (*1*) and Hayes et al. (*8*) reported what they considered to be a "genetic linkage" between susceptibility to black chaff and the mature-plant type of resistance to stem rust derived from Hope and H–44, but believed that their data indicated that the linkage could be broken.

Bamberg (*2*) stated that Hope and H–44 are among the varieties most susceptible to black chaff and that there is danger of the disease becoming epidemic should selections obtained from crosses with these varieties become widely grown. This opinion is typical of many that are expressed in the literature.

Greaney (*3*, *4*) found that, in a rust-resistant descendant of H–44, applications of sulphur dust prevented the development and spread of a "bacterial disease of wheat called black chaff."

More recently Hagborg (*5*) has reported that black chaff is a composite disease. He reached the conclusion that one form of the disease, which he designated as "internodal melanism," is the result of a physiologic disorder caused by exposure of the plants to unfavorable conditions. This conclusion was reached largely because the disorder had been produced in certain rust-resistant varieties by subjecting the plants to high temperatures in a humidity chamber.

In a preliminary article (*11*) the writer concluded that at least a part of the so-called black chaff disease of Hope and H–44 was the result of infection by stem rust. The present paper contains the detailed experiments and observations upon which those conclusions were based.

DESCRIPTION OF BROWN NECROSIS

Brown necrosis first appears in the form of brown or purplish-brown blotches of various sizes on the culms, peduncles, glumes, and rachises, and occasionally on the awns and leaf sheaths. On the stems, just below the nodes, the lower margins of the blotches usually are more or less distinctly striated. On the sheaths, the blotches are of less common occurrence and, when they do occur, are often in the form of halos surrounding chlorotic areas. The blotches usually appear during the second week after heading and shortly thereafter change to dark brown or nearly black accompanied by necrosis of many of the plant cells. The discolored areas usually approach their maximum development in a given variety of wheat within a few days after their first appearance, following which there is little further development within that variety except on the later maturing culms. Upon first appearance, brown necrosis can usually be identified by its purplish-brown color, whereas lesions of those diseases with which brown necrosis is most easily confused start as water-soaked areas. The appearance of reddish-purple spots around rust pustules should not, however, be confused with brown necrosis. Furthermore, such spots are not an indication of rust resistance, as they often appear in numerous susceptible varieties of wheat that are characterized by purple pigment even in healthy culms.

EARLY OBSERVATIONS AND EXPERIMENTS WITH BROWN NECROSIS

The condition designated in this paper as brown necrosis was first observed by the writer in 1915 on the stems of the rust-resistant emmer varieties Vernal and Yaroslav. In years following, it was observed also on rust-resistant selections from crosses of these varieties and also on Acme durum and rust-resistant selections from crosses with it. The condition was at first confused with anthracnose (*Colletotrichum graminicolum* (Ces.) Wils.), a disease referred to by Wilson (*13*) the preceding year. Later observations, however, showed that it was not identical with that disease. Following these observations, numerous attempts to isolate a causal organism and to transfer the disease from stem to stem by the puncture method met with failure. Also, attempts to control the disease by various seed treatments were fruitless. It soon became apparent that the condition was closely associated with resistance to stem rust. In the belief that the association might be the result of genetic linkage, numerous attempts were made to break the linkage by backcrossing to resistant varieties. All of these efforts gave negative results.

Further observations showed that brown necrosis did not spread from infection centers within a susceptible variety. Large fields of Hope wheat often have remained practically free from the necrotic condition, which developed profusely on the same variety grown in nursery rows adjacent to rust-susceptible varieties. Also, in common with stem rust, brown necrosis usually has developed more profusely on the north sides of the culms than on the other sides. (In the Texas Gulf coast in 1936 and 1937, however, the heaviest stem rust infection and brown necrosis developed on the south sides of the culms.) All of these observations give support to the belief that the brown pigmentation may follow an arrested infection of stem rust, the inoculum of which comes from rust-susceptible varieties. Nevertheless, numerous attempts to produce the pigmentation in the plants after they were fully headed, by spraying with suspensions of rust spores in water, gave only negative results.

EXPERIMENTAL PRODUCTION OF BROWN NECROSIS

In the summer of 1932, at Redfield, S. Dak., several plants of Hope wheat growing in the field were inoculated hypodermically at different stages of growth with suspensions of stem rust spores in distilled water, in an effort to determine at what stage of development the mature-plant type of rust resistance becomes effective. It was found that when the culms were inoculated while the plants were in the early boot stage a light infection of rust developed. When inoculation was delayed until just before the head emerged from the boot, little or no rust developed but a profuse development of brown necrosis resulted. It was at first thought that this might be due to contamination of the rust inoculum with some other organism that was responsible for the brown necrosis. It was noted, however, that rust and brown necrosis did not develop simultaneously on the same culms. Further experiments were delayed because of crop failures in 1933 and 1934.

The experiment was repeated in 1935 on the agronomy plots at University Farm, St. Paul, Minn. Here again, a light infection of stem rust developed on the Hope plants when inoculated prior to the late boot stage, but when inoculated just before the spikes emerged from the boots, brown necrosis developed instead of rust. However, since brown necrosis also developed in certain other material in all parts of the nursery at about the same time, it could not be determined definitely whether its occurrence on the inoculated plants had resulted from the inoculations or from natural infection that spread from surrounding material.

The study was resumed at the Texas Agricultural Experiment Station, College Station, Tex., in the spring of 1936. Twelve culms each of Hope and Kota X Webster (H-151) growing in the wheat nursery were inoculated hypodermically with a suspension of urediospores of stem rust of wheat collected from three widely separated infection centers on the experiment station plots. This rust was later identified by pathologists at the University of Minnesota as races 11 and 17. Hope wheat, in the seedling stage, is susceptible to both of these forms, while H-151 is moderately resistant in both seedling and mature-plant stages.

The inoculations were made by injecting the inoculum into the boots just before the spikes emerged. At the same time, the leaf sheath of the second leaf from the top was gently drawn away from the stem and a small quantity of inoculum was inserted between it and the stem. Twelve other culms each of Hope and H-151 were treated in like manner with boiled distilled water. A third set of culms in the same stage of development was left untreated. Thirteen days later the 12 culms of H-151 that had been inoculated with stem rust spores were covered heavily with small pustules of stem rust on the stems, necks, sheaths, and glumes, while the untreated culms and those that had received injections of boiled distilled water were entirely free from both stem rust and brown necrosis.

The inoculated culms of Hope were free from rust pustules on all parts exposed to sunlight but had a profuse development of brown necrosis on the glumes and necks and on the stems just below the nodes where the rust inoculum had been inserted under the leaf sheaths. On a few of the inoculated culms of Hope, fairly normal rust pustules, but no brown necrosis, developed beneath the sheaths where the tissues were not exposed to direct sunlight. The untreated culms of Hope, as well as those receiving injections of boiled distilled water, remained free from both rust and brown necrosis. Typical specimens of treated plants are shown in figures 1, 2, and 3. It is noteworthy that no other brown necrosis could be found anywhere else in the nursery at that time except in one row of Hope wheat that was located only 2 feet from the first-observed infection center of stem rust in the nursery. In this row of Hope, every culm in which the stem was

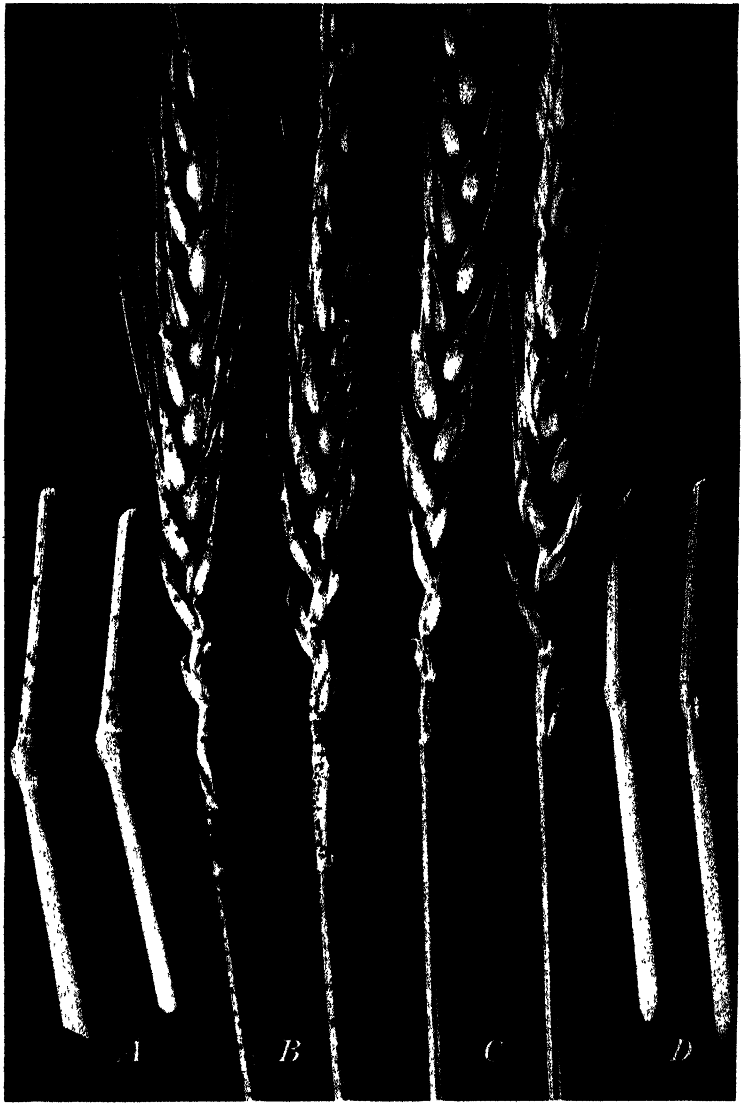

FIGURE 1.—Effect of hypodermic inoculation of Kota × Webster (H–151) in the late boot stage with urediospores of stem rust. *A* and *B*, Stems and peduncles photographed 15 days after inoculation. Note profuse development of stem rust, but no brown necrosis. *C* and *D*, Stems and peduncles injected with boiled distilled water.

exposed below the upper node showed some brown necrosis and many of the necks and glumes also were affected but no stem rust pustules could be found. An adjoining row of H–151 was heavily infected

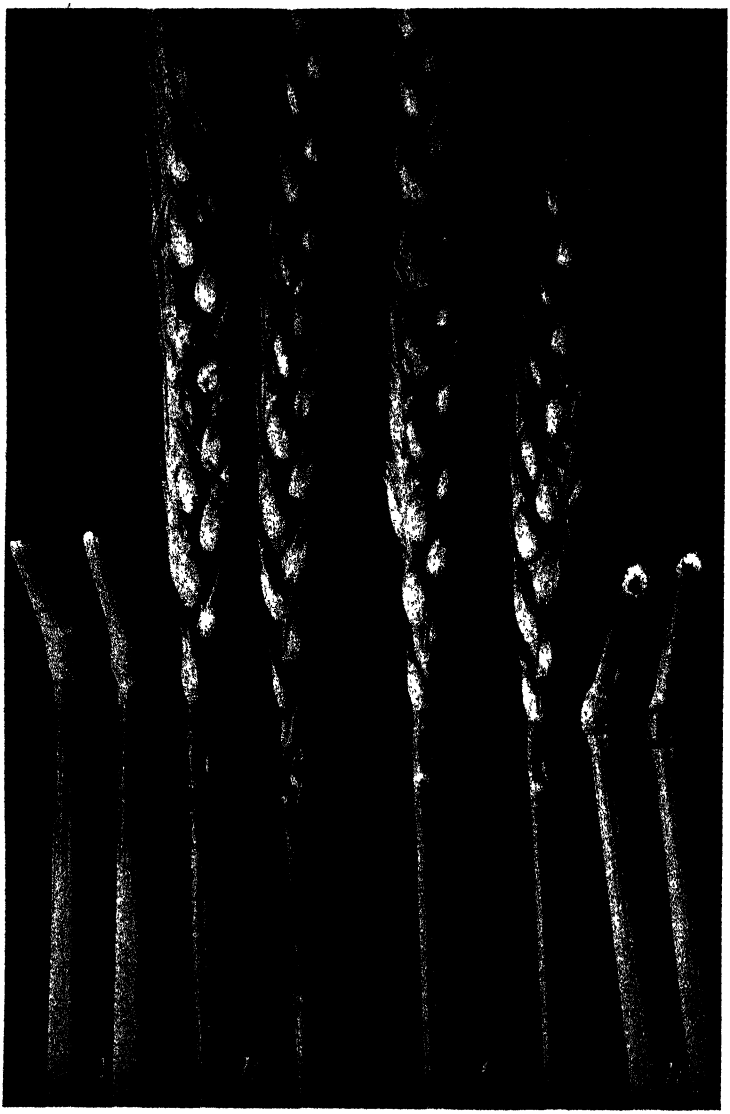

FIGURE 2.—Effect of inoculating Hope wheat in the late boot stage with urediospores of stem rust. *A* and *B*, Stems and peduncles of Hope wheat photographed 15 days after inoculating. Note the profuse development of brown necrosis, but no rust pustules. *C* and *D*, Stems and peduncles of Hope wheat checks injected with boiled distilled water.

with stem rust from the ground clear up to the tips of the awns but developed no brown necrosis. In another part of the nursery, about 100 feet south of the above-mentioned rust-infection center, no brown necrosis whatever could be found on a row of Hope wheat, and likewise there was no stem rust on an adjoining row of H–151. Thus there appeared to be a direct relationship between the presence of stem rust on the moderately rust-resistant variety H–151 and the presence of brown necrosis on adjoining rows of Hope wheat. Figure 4 shows typical stems of Hope and H–151 from the above-described locations.

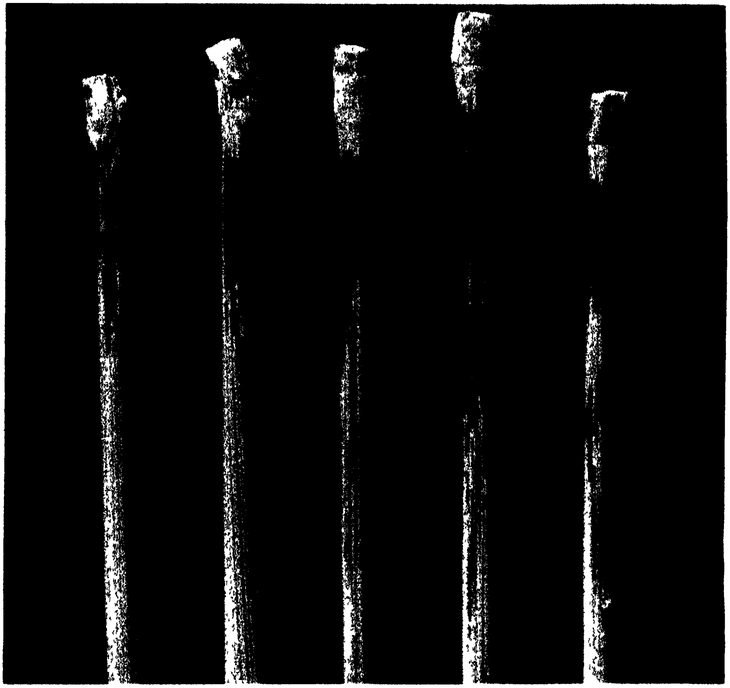

FIGURE 3.—Typical stems of Hope wheat inoculated with stem rust at the late boot stage by injecting inoculum underneath the leaf sheaths; the sheaths have been stripped off to show the results of the inoculation. Brown necrosis developed profusely between the nodes and the tops of the leaf sheaths where the stems were exposed to direct sunlight. Considerable stem rust, but only a trace of brown necrosis, developed underneath the sheaths where the tissues were not exposed to direct sunlight.

The material was photographed about 10 days after the plants headed, which was approximately 10 days before rust infection became uniformly distributed in the nursery. It is recognized that this experiment does not prove that organisms other than *Puccinia graminis tritici* Eriks. and Henn. or other conditions might not cause brown necrosis, although the relationship to rust is strongly suggestive.

OBSERVATIONS IN NURSERY PLOTS

An excellent opportunity to observe the relationship between stem rust infection and brown necrosis was also afforded in the replicated wheat nursery at College Station, Tex., in 1936. This nursery consisted of four replications of 18 rust-resistant selections from a Hope × Mediterranean (T. S. 3015–63) cross, with Denton, which is rust susceptible, and a bulk population of Hope × Mediterranean (T. S. 3015–63) used as checks. The wheats were grown in triple-row groups with rows running northeast and southwest and replicated four times. The planting order was in the form of a modified Latin square. Although the planting order was partly randomized, Denton and the bulk Hope × Mediterranean fell side by side in all four replications. Since the Denton variety and many of the Hope × Mediterranean plants were susceptible to stem rust, there occurred in each replication a block of six rows of wheat in which susceptible plants occurred that served as a center from which rust spores were being disseminated after the susceptible plants had become infected. The spread of stem rust from infection centers under the conditions of southern Texas is almost entirely toward the north or northwest. It was decided, therefore, to compare the amount of brown necrosis in a given wheat selection in replications where it was grown on the northwest side adjacent to the rust-susceptible block with the amount in the same selection in replications where it was grown at various distances from susceptible blocks. The comparisons (table 1) are based on the percentage of infected peduncles in 100 culms in the center row of a three-row group. It will be observed that in every selection the percentage of culms on which brown necrosis developed was highest in the replication grown adjacent to the rust-susceptible block.

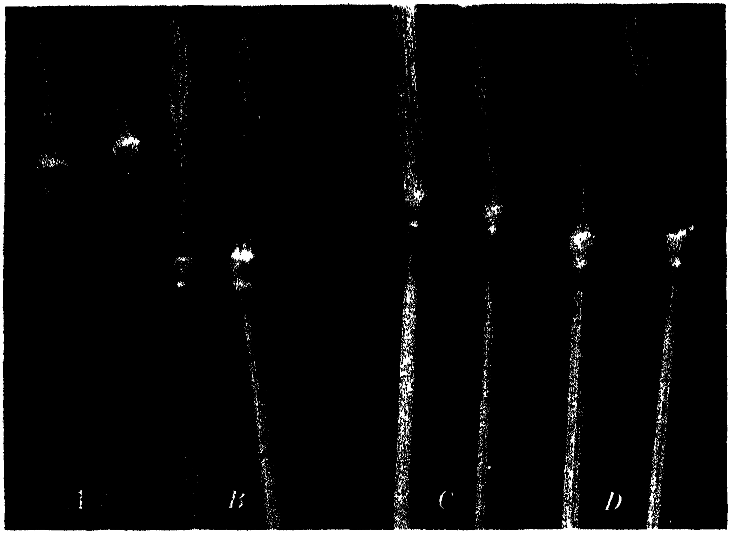

FIGURE 4.—Effect of natural rust infection on Hope and Kota × Webster (H-151): *A*, Stems of Hope wheat grown within 1 foot of an early infection center of stem rust; *B*, stems of Kota × Webster (H-151) grown in a row adjacent to *A*; *C*, stems of Hope wheat grown 100 feet south of the same infection center; *D*, stems of Kota × Webster grown in a row adjacent to *C*. Photographed about 10 days after heading.

TABLE 1.—*Effect of distance from source of rust inoculum on development of brown necrosis*

Selection No.	Culms that developed brown necrosis in indicated row						
	Next to rusted block	In replications					
		1	2	3	4	Average	
						Actual	Comparative[1]
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
41-22-1	73	--	19	13	28	20.0	27.4
41-8-3	57	17	--	21	37	25.0	43.9
41-57-3	33	21	[2] 29		15	21.7	65.8
41-27-3 [3]	41	33	[4] 36	27	--	32.0	78.0

[1] In terms of percentage of plot next to rusted block.
[2] Next to segregating guard row.
[3] Segregating for rust reaction.
[4] 5 feet north of segregating guard row.

OBSERVATIONS IN FIELD PLOTS

Another opportunity to observe the relation of rust inoculum to the development of brown necrosis was afforded by conditions existing in the field plots of wheat at College Station, Tex., in 1936. These plots consisted of 11 rust-resistant selections from the Hope × Mediterranean cross (T. S. 3015–63) grown in comparison with an unselected population of this cross. The plots were separated by alleys 2 feet wide, with a row of Mediterranean (T. S. 3015–105), which is very susceptible to stem rust, planted in the center of each alley. Several infection centers of stem rust had appeared in the Mediterranean wheat in the alleys by the time the Hope × Mediterranean selections started heading. These centers were marked as soon as they were found. Shortly before the wheats ripened, the development of brown necrosis on the Hope × Mediterranean selections was found to be much heavier adjacent to the rust-infection centers than elsewhere in the plots. The contrast was so distinct that one could stand at a considerable distance and locate the infection centers in the guard rows by the dark spots in the adjoining plots.

STAGE OF DEVELOPMENT OF THE HOST AT THE TIME OF RUST INOCULATION AND DEVELOPMENT OF BROWN NECROSIS

Heavy infections of stem rust often occur on susceptible varieties in the spring wheat area with but little brown necrosis developing on most of the rust-resistant types derived from Hope and H–44 in the same nursery. Under such conditions, it has been observed by the writer in several years that the later maturing rust-resistant strains developed more brown necrosis than the earlier maturing strains. This observation suggested that brown necrosis follows rust infection that takes place at the time of heading or a few days thereafter. An experiment was therefore conducted to determine whether such is the case (table 2). Heads and necks of Hope and Tenmarq in four different stages of development were inoculated with stem rust. The four stages were (1) early boot, (2) late boot, (3) early heading, and (4) about 10 days after heading. In the early and late boot stages, the inoculations were made by hypodermic injections into the boot. In the two later stages, they were made by wrapping the heads and necks in sterilized gauze saturated with a suspension of rust spores in boiled distilled water. A wrapping of cellophane was then placed over the gauze to prevent drying. The heads were unwrapped after 18 hours. The rust inoculum used, which was the same in all cases, was collected in the field from three of the first infection centers that developed in the agronomy plots at College Station and was identified by pathologists at the University of Minnesota as races 11 and 17. To this was added inoculum of physiologic race 21 from greenhouse cultures. Hope wheat, in the seedling stage, is susceptible to all three of these races. The results of these inoculations are shown in table 2.

TABLE 2.—*Effect of inoculating Hope and Tenmarq wheat with a composite inoculum of Puccinia graminis tritici, races 11, 17, and 21, at different stages of development*

Variety	Stage of host at time of inoculation	Reaction	
		Rust	Brown necrosis
Hope	Early boot	Heavy	None [1]
Tenmarq		do	Do
Hope	Late boot	Trace	Heavy.
Tenmarq		Heavy	None.
Hope	Early head	None	Light.
Tenmarq		Heavy	None
Hope	Late head (about 10 days after heading).	None	Do.
Tenmarq		Heavy	Do.

[1] No brown necrosis at 15 days but a heavy development on the spikes and necks about 5 days later.

A heavy infection of stem rust, but no brown necrosis, developed in Tenmarq from inoculations at all four stages.

A heavy development of rust on the necks, glumes, and awns of Hope appeared by the fifteenth day after inoculating in the early boot stage, but no brown necrosis appeared then. However, about 5 days later the rust pustules were surrounded by brown necrotic areas and development of rust pustules ceased.

In Hope plants inoculated in the late boot stage, a trace of small rust pustules began to appear on some of the glumes of the lower spikelets 12 days later. On the thirteenth day brown necrosis started developing around these pustules, after which there was no further development of pustules, and the brown necrosis continued developing for 2 days until it extended over the necks, glumes, rachises, and awns.

No rust pustules appeared on Hope plants inoculated in the early heading stage, but a light development of brown necrosis appeared on about the tenth day after inoculating and by the twelfth day the development was fairly heavy.

Where Hope was inoculated about 10 days after heading, no stem rust, brown necrosis, or white necrotic flecks developed. The results of these inoculations show that brown necrosis in Hope wheat developed, after an exposure to stem rust infection, while the host tissues were still succulent and comparatively young and before the maximum of mature-plant resistance to the rust had been acquired.

REACTION TO STEM RUST IN VARIETIES HAVING DIFFERENT TYPES OF RESISTANCE

An experiment was conducted to determine (1) whether other varieties carrying the Hope type of resistance to stem rust would produce a like development of brown necrosis and (2) whether varieties having other types of resistance would give the same or different reactions. This experiment was conducted in a small plot at some distance from other wheats in which natural infection by stem rust did not occur until about 20 days after the artificial inoculations were made.

The wheat varieties in this experiment were Hope and Hope × Reliance (N. N. 1131), which carry a distinct mature-plant type of resistance derived from emmer; Kota × Webster (H–151) and Kota × Webster (H–231), which carry a distinct morphologic type of resistance (also of a mature-plant nature) associated with a long incubation period; Kanred and Thatcher, which carry a high degree of seedling resistance to several physiologic races of stem rust; *Triticum timopheevi* Zhuk., which carries a high degree of both seedling and mature-plant resistance; and Denton, Mediterranean (T. S. 3015–105), Kawvale, Cheyenne, and Quivira, which probably are susceptible to most physiologic races of stem rust in both the seedling and mature-plant stages.

Three plants of each of the above varieties were inoculated hypodermically while in the late boot stage of development, part of the inoculum being injected between the stem and sheath of the second leaf from the top of the plant and part into the boot. The inoculum used was from the same sources as in the previous experiments. On the fifteenth day after inoculating, the results were recorded (table 3).

After these notes had been made, the plants were observed carefully at intervals of about 5 days or less throughout the filling and ripening periods, and the changes that occurred are shown in footnotes in table 3.

TABLE 3.—*Effect of inoculating varieties of wheat with composite inoculum of Puccinia graminis tritici, races 11, 17, and 21, at the late boot stage of development*

Rust-reaction class	Variety	Reaction 15 days after inoculation	
		Rust pustules	Brown necrosis
Photologic resistance [1]	Hope	None	Heavy
	Hope × Reliance (N. N 1131)	do	Do.
Morphologic resistance.	Kota × Webster (H–151)	Heavy (small pustules)	None.
	Kota × Webster (H–231)	do	Do.
Seedling resistance	Kanred	Light (medium size)	Do.
	Thatcher	None [2]	Do.[3]
Seedling and mature-plant resistance.	*Triticum timopheevi*	do	Do [4]
Susceptibility	Denton	Heavy	Do.
	Mediterranean (T. S. 3013–105)	do	Do.
	Kawvale	do	Do.
	Cheyenne	do	Do.
	Quivira	do	Do.

[1] See p. 817.

[2] A fairly heavy infection of stem rust on both inoculated and uninoculated culms developed about 35 days after inoculation.

[3] A small brown blotch developed below 1 of the nodes and another on 1 glume of 1 of the inoculated culms of Thatcher, but these were not typical brown necrosis.

[4] Smoky-appearing areas (not brown necrosis) developed on the leaf sheaths just above the basal swellings. These also developed on uninoculated culms later.

In the Hope and Hope × Reliance wheats some rust pustules appeared beneath the sheaths of the inoculated plants where the tissues were not exposed to direct sunlight. Brown necrosis did not develop beneath the sheaths in either the inoculated or the uninoculated plants. Brown necrosis started developing on the checks about 25 days after the inoculations were made, or 10 days after the first appearance of rust pustules on the inoculated susceptible varieties.

In this experiment and in those recorded previously the typical brown necrosis occurred only in Hope wheat and in some related strains having a similar type of mature-plant resistance to stem rust.

BROWN NECROSIS AS AN INDICATOR OF MATURE-PLANT RESISTANCE

In wheat breeding, particularly in backcrossing, it is important to be able to determine, prior to the blooming period, which plants carry the factor for mature-plant resistance to stem rust. This cannot be determined by seedling reactions. Eighty-one plants of F_2 generation of an H-44 × Marquis cross were inoculated hypodermically with a mixture of rust races 11, 17, and 21 just before coming into head. Four of these plants later developed root rot and were discarded. Fifty-four of the seventy-seven remaining plants developed brown necrosis within 15 days and were later found to carry a high degree of mature-plant resistance to stem rust. Eighteen other plants proved to be completely susceptible to stem rust and developed no brown necrosis. The five remaining plants showed a moderate degree of mature-plant resistance to stem rust of a morphologic type resembling that found in the Kota × Webster selections H-151 and H-231. These five plants were also entirely free from brown necrosis. The data are given in table 4.

TABLE 4.—*Reaction of F_2 plants of an H-44 × Marquis cross to hypodermic inoculations, at the late boot stage, with a composite inoculum of Puccinia graminis tritici, races 11, 17, and 21*

Reaction of plants to brown necrosis	Total plants	Plants showing indicated reaction to stem rust		
		Photologic resistance	Susceptibility	Morphologic resistance
	Number	*Number*	*Number*	*Number*
Necrotic	54	54	0	0
Not necrotic	23	0	18	5
Total	77	54	18	5

The fact that the photologic type of resistance, associated with brown necrosis, occurred in 54 plants out of a total of 77 suggests that it is inherited as a simple dominant. The inheritance of the morphologic type of mature-plant resistance, which occurred in five plants, could not be determined from the meager data. The type of mature-plant resistance, which amounts to near immunity, was in all cases associated with brown necrosis. In most cases it was found that this identification could be made before the later heads had reached the blooming stage, and hence in sufficient time to allow for crossing.

DISCUSSION

The experiments and observations presented herein show that under certain conditions dark-pigmented areas, to which the term "brown necrosis" is applied, may develop on the internodes, peduncles, glumes, and rachises of certain rust-resistant varieties of wheat following inoculation with *Puccinia graminis tritici*. This reaction appears to be confined to wheats having a specific type of mature-plant resistance, such as Hope, H-44, and certain other varieties. Since it has been found by Hart and Zaleski (*7*) that Hope wheat has a type of resistance dependent on high light intensity, and by the present writer that brown necrosis as well as one type of resistance is dependent on high light intensity, it is suggested that the term "photologic resistance" be applied to this type of resistance, which is very likely of a physiologic nature. The other type of resistance that segregates out of H 44 crosses appears to be of a "morphologic" nature as described by Hursh (*9*) and Hart (*6*). In the experiments and observations presented here, brown necrosis appeared when the tissues of the host were inoculated with stem rust spores before photologic resistance was fully developed.

Brown necrosis bears a resemblance to the lesions produced by several other disease organisms, particularly *Bacterium translucens* var. *undulosum*. It appears from an examination of the literature that brown necrosis has been confused with the symptoms of other diseases by the writer [6] (*10*) as well as by others. The writer's report [6] of having broken the association between resistance to stem rust and susceptibility to the so-called black chaff was based on a study of F_2 plants and F_3 lines from natural crosses between certain Kota × Webster selections and sister strains of Hope and H-44. From unpublished data, it now appears that the Kota × Webster selections carry a factor that is either epistatic to, or inhibits the development of, the photologic type of resistance. Consequently, this type of resistance is expressed in only approximately one-sixteenth of the F_2 plants of such crosses. All of the other resistant plants of the F_2 generation of such crosses owe their resistance to factors other than the factor for photologic resistance. The type of resistance found in the Kota × Webster selections, as well as one type that segregates out of H-44 crosses, is not associated with brown necrosis. Therefore, a large majority of the rust-resistant segregates from such crosses are free from brown necrosis. This type of segregation would seem to account for the belief that the association between resistance to stem rust and susceptibility to black chaff (brown necrosis) is not complete.

The field experiments reported by Waldron (*12*), Ausemus (*1*), Hayes et al. (*8*), and Bamberg (*2*), in which many of the hybrid strains having the Hope type of resistance appeared to be very susceptible to black chaff, were conducted in nurseries where stem rust was present and where brown necrosis might thus be expected to appear. Dusting with sulphur, which controlled an unidentified disease called black chaff in rust-resistant wheats in experiments by Greaney (*3*, *4*), also should control rust and prevent accompanying brown necrosis. The description and illustrations of the "internodal melanism," "which seemed to be of non-parasitic origin," reported by Hagborg (*5*) in derivatives of H-44 and Pentad wheats are typical of brown necrosis.

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[6] McFadden, E. S. See footnote 3.

The confusion of brown necrosis with other diseases appears to have been universal. Although several investigators appear to have entertained doubts concerning the specific organism that caused the discoloration, most of them seem to have held the opinion that it was a disease of bacterial origin.

The inoculation experiments and observations reported herein suggest that brown necrosis might be expected to appear in epidemic proportions under field conditions when wheat varieties carrying the factor for potential photologic resistance to stem rust are exposed to heavy infections by stem rust spores while the spikes are emerging and the stems still elongating. Under normal field conditions in the spring wheat areas of the United States and Canada, inoculum seldom would be sufficiently abundant at that stage to produce a heavy development of brown necrosis unless the rust-resistant variety were grown in close proximity to an earlier maturing, rust-susceptible variety. It appears, therefore, that the fears repeatedly expressed by various workers that the black chaff disease of Hope and H–44 might become of a serious nature should wheats derived from these varieties become widely grown are entirely ungrounded. On the contrary, it would be expected that should wheats having the photologic type of resistance to stem rust become widely grown stem rust inoculum would be greatly diminished, and in consequence, the so-called black chaff disease (brown necrosis) would practically disappear.

SUMMARY

When varieties of wheat such as Hope and H–44, which have a specific type of mature-plant resistance to stem rust, were artificially inoculated with stem rust spores, or exposed to natural infection, dark-brown necrotic areas were produced around the points of infection. The term "brown necrosis" is suggested for this discoloration to distinguish it from several diseases of wheat which it resembles.

A critical review of the literature indicates that brown necrosis has often been confused with several wheat diseases, usually the bacterial black chaff disease caused by *Bacterium translucens* var. *undulosum*.

Two distinct types of mature-plant resistance to stem rust were found in segregates from an H–44 × Marquis wheat cross. These types are designated as "photologic" and "morphologic" resistance, the former being so termed because it is expressed only under high light intensity. Brown necrosis occurred only in plants carrying the photologic type of resistance, which offers a possible explanation of previous reports of close but incomplete "linkages" and "associations" between resistance to stem rust and susceptibility to the so-called black chaff.

Inoculation experiments with F_2 plants from a cross between H–44 and Marquis wheats suggest the feasibility of using the brown necrosis reaction as an indicator for identifying plants that carry the factor for photologic resistance at an early stage of their development. Since this determination can be made prior to blooming, it would simplify breeding for rust resistance by the backcross method.

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ROOT COMPOSITION AND TOP DEVELOPMENT IN LARGE PECAN TREES HEADED TO VARIOUS DEGREES OF SEVERITY IN TOP WORKING[1]

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INTRODUCTION

An important phase of pecan (*Carya pecan* (Marsh.) Engl. and Graebn.) orchard development and management is the top working of native or undesirable trees to desirable varieties. Top working is practiced on trees of all sizes but is usually confined to trees having trunks less than 12 inches in diameter. Many different methods of top working pecan trees have been practiced, but not all successfully. In many instances the heading back of the top has been severe; the large wounds made have failed to heal and have become badly infected with wood-decaying fungi, with the result that the tops have been only partially rebuilt. The main incentive for severe topping is that it requires fewer buds or grafts and less time and expense to top-work a tree when it is cut back heavily than when it is cut back less severely. To be satisfactory, a method of top working must be biologically successful, i. e., the tree should be rebuilt to normal size and vigor; furthermore, the cost must be low.

The experiment herein reported was designed to determine the effects of different degrees of heading large pecan trees on their subsequent development after being top-worked to desired varieties.

MATERIAL AND METHODS

EXPERIMENTAL BLOCK

The experimental block, consisting of 9 rows of 32 trees each, excepting a few spaces where the trees had previously died, was located in the orchard of H. G. Lucas, in the valley of the Pecan Bayou, near Brownwood, Tex. The trees were 43 years old at the beginning of the experiment, having been grown from orchard-planted seeds and seedling trees planted in 1888. The trees were 40 feet apart on the square system. The average diameter of the tree trunks was about 12 inches in March 1931.

DEGREES OF SEVERITY OF HEADING

No heading.—One row of trees was left as checks without being cut back or top-worked (fig. 1, *A*).

Headed to stumps.—The trees in two rows were cut off approximately 3½ feet above the ground (fig. 1, *B*). Sloping wounds were made with the exposed surface facing the north so as to prevent drying out as much as possible. These wounds were from 12 to 18 inches in diameter except on a few small trees.

Headed to first forks.—The trees in two rows were cut off 8 to 10 inches above the first forks (fig. 1, *C*). The wounds were made at

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[1] Received for publication July 11, 1938.

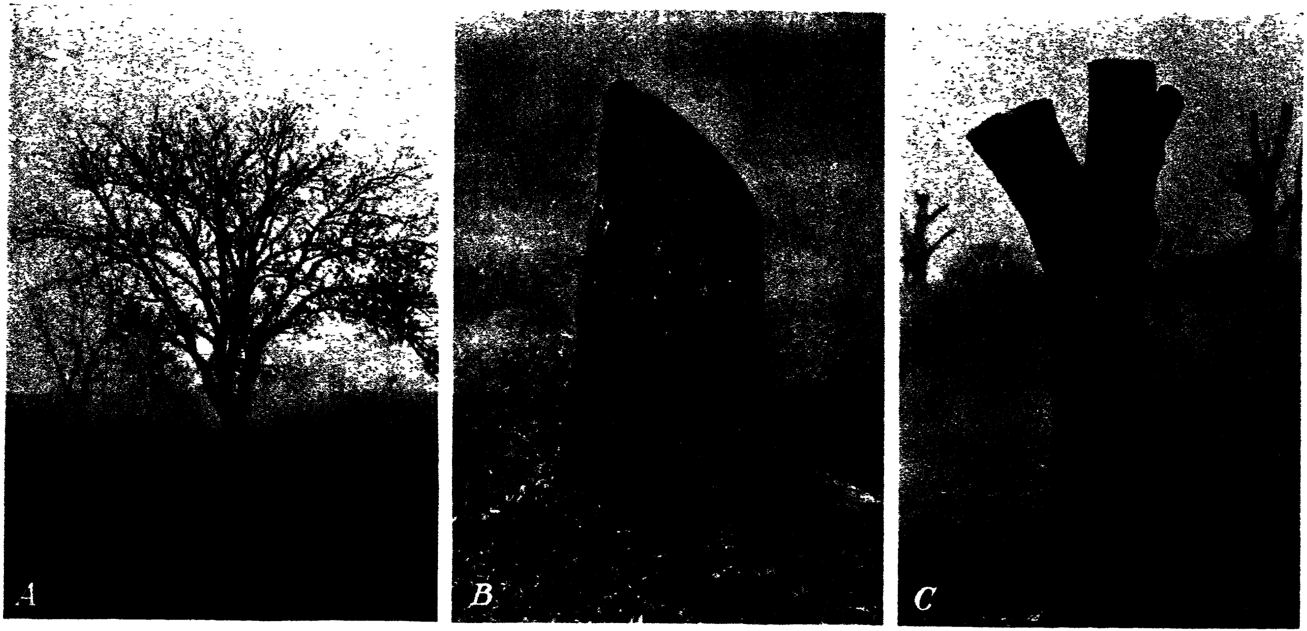

Figure 1.—Typical trees headed to different degrees of severity: *A*, Check tree, no heading; *B*, headed back to stump; *C*, headed to first forks. Note large wounds 6 to 8 inches in diameter.

right angles to the branches at first, and later cut to a slope after new shoot growth was sufficiently advanced. These wounds averaged 6 to 8 inches in diameter.

Headed to second forks.—Trees in two rows were cut off about 8 or 10 inches above the second forks (fig. 2, *A*). The wounds were made and treated in the same manner as in the trees headed to the first forks. These wounds averaged about 6 inches in diameter.

Headed to third forks.—Alternate trees in two rows were headed just above the third forks (fig. 2, *B*). The wounds averaged about 3 or 4 inches in diameter and were made and treated as in the trees headed to first forks.

Headed to fourth forks.—Alternate trees in two rows were headed just above the fourth forks (fig. 2, *C*). The diameter of the cuts averaged 2½ to 4 inches, most of them being not over 3 inches, and were made and treated as in the trees headed to first forks.

SUBSEQUENT PROCEDURE

The heading was all done in March 1931, and all wounds were protected by pruning paint. About one-half of the trees were grafted in the spring of 1931; the others were budded in late summer of that year. Trunk-circumference measurements and yield records of all trees were taken each year. A record of the cost of top working the trees in each treatment was also kept, as well as notes on the healing of wounds and infection by wood-rot fungi. However, this paper is concerned principally with the seasonal composition of the roots of trees and the growth responses following the different degrees of severity of heading.

Root samples were obtained from trees under each treatment at intervals from October 2, 1931, to January 23, 1934, and were analyzed for dry matter, sugars, starch, hemicellulose, and nitrogen. Three samples from check trees were taken after January 23, 1934. The data for the composition of roots of the check trees indicate the nutritional condition of normal trees under the conditions of the experiment.

ANALYTICAL METHODS

Sampling

Roots as uniform as possible but ranging between 5 and 20 mm in diameter were taken from trees in each treatment. The roots from each tree were washed free of adhering soil, wiped with a cloth towel, and allowed to dry in the air for 30 minutes. A composite sample of approximately 300 g was then made from the lots of roots. After the ends from each length of root had been clipped off, the remaining portion was cut into thin sections with pruning shears, placed in a covered soil-moisture can, and thoroughly mixed by shaking.

Two tared glass-stoppered weighing bottles were filled from this composite sample, weighed before any appreciable moisture loss occurred, and then saved for the determination of dry matter.

Two 50-g samples were weighed and transferred to pint fruit jars containing 250 ml of boiling 95-percent ethyl alcohol and a small amount of calcium carbonate. The covers were placed loosely on the jars and the boiling was continued for half an hour, after which the jars were removed from the water bath and sealed while hot. With this material this procedure is calculated to give a sample preserved in approximately 80-percent alcohol by weight.

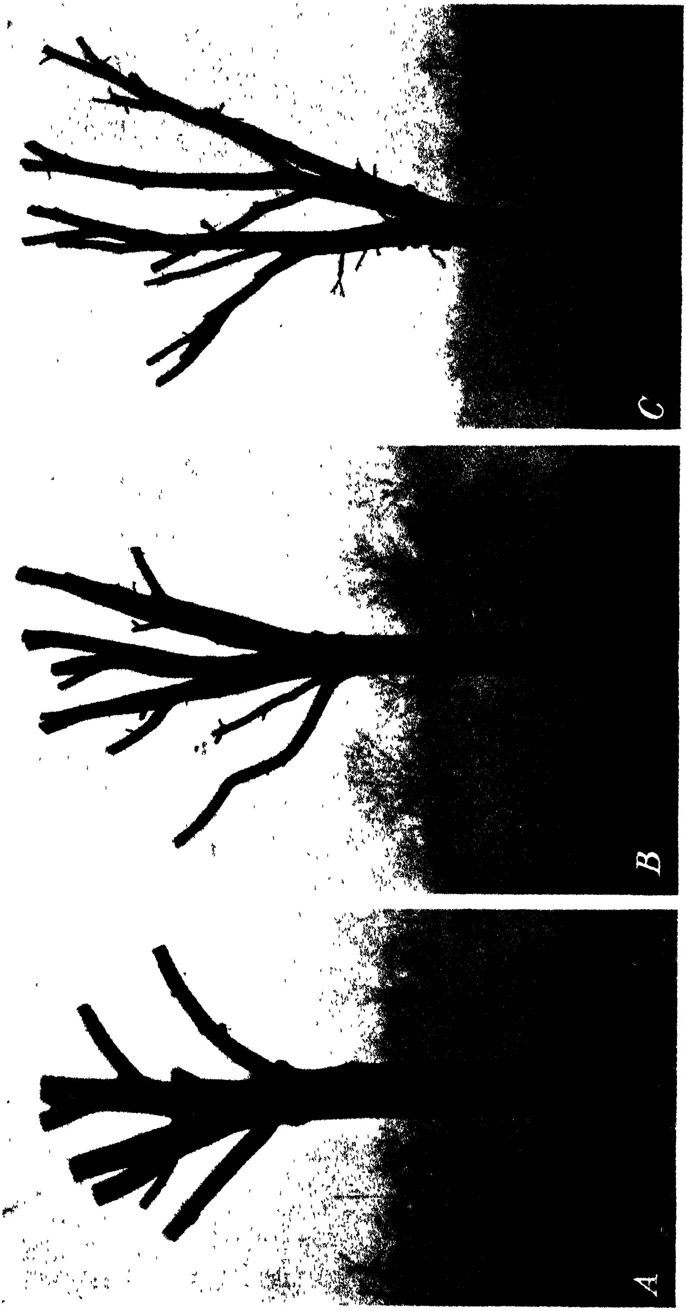

FIGURE 2.—Typical trees headed to (*A*) second forks, (*B*) third forks, (*C*) fourth forks. Note the small wounds, which heal easily, and the large framework for placing of buds and grafts.

DETERMINATION OF DRY-MATTER CONTENT

The samples were dried in a vacuum oven at 80° C. and at a pressure of less than 1 inch of mercury. Since preliminary tests at 6, 12, 18, and 24 hours of drying showed that practically constant weight was attained in 6 hours, the samples were dried for two 6-hour periods and weighed at the end of each. The difference between these two weights was always well under 0.5 percent of the total moisture loss.

DETERMINATION OF SUGARS

Each alcohol-preserved sample was filtered and the solid portion transferred to an alundum extraction thimble 45 by 127 mm. It was then extracted for 24 hours in a large-size Soxhlet apparatus with the filtrate and alcoholic washings in the extraction flask. The residue was removed, dried in an air oven at 100° C. for 4 hours, and ground in a drug mill, care being taken that no significant loss of material occurred. The ground residue was again extracted for 24 hours with the same alcoholic solution. Preliminary tests had shown that the grinding and second extraction were necessary in order to insure complete extraction of sugars. The final residue was dried in an air oven at 100° for 4 hours, weighed, and saved for the determination of starch.

The alcoholic extract was concentrated in vacuo at a temperature below 55° C. to a volume of a few milliliters. This was taken up in water and filtered into a 250-ml volumetric flask. After adding 25 ml of saturated neutral lead acetate solution to the filtrate and washings, the mixture was made up to volume. The mixture was filtered through a dry filter, and the excess lead was precipitated from the clarified solution by means of anhydrous disodium phosphate. After being filtered through a dry filter, the solution was used for the determination of reducing and nonreducing sugars.

Reducing sugars were determined in 25-ml aliquots by the Munson-Walker method, the amount of reduced copper being determined by the volumetric permanganate method (*2, pp. 190–192* [*34–39*]).[2] Results were calculated as dextrose.

For nonreducing sugars, hydrolysis was effected with hydrochloric acid at room temperature by the procedure given in Official Methods (*2, p. 189* [*28*]). Results were calculated as sucrose.

DETERMINATION OF STARCH

The dried and weighed residue from the alcoholic extractions was ground once more in the drug mill, and samples were weighed from this for the individual starch determinations.

Starch was determined by direct acid hydrolysis as outlined in Official Methods (*2, p. 119* [*21*]) except that the preliminary washing with water was omitted since the material was already sugar-free and it was desired that any dextrin which might be present should be included in the result. The starch by the diastase method was subtracted from the starch by direct acid hydrolysis for corresponding samples, and the values thus obtained are referred to as hemicellulose. These values include the carbohydrate bodies that are not hydrolyzed by diastase, such as dextrins and pentosans, but are hydrolyzed by dilute mineral acids.

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[2] Italic numbers in parentheses refer to Literature Cited, p. 841.

Starch was also determined by the diastase method essentially as outlined in Official Methods (*2*, *p. 120* [*23*]). A freshly prepared 0.5-percent solution of takadiastase in water was used instead of the malt extract. The preliminary washing of the sample was again omitted for the same reasons as before.

In both cases the data for the starch content were finally calculated back to the original alcohol-preserved sample.

DETERMINATION OF TOTAL NITROGEN

The dried residue from the determination of dry matter was ground in a drug mill, and 2-g samples were weighed out for nitrogen determinations. Total organic nitrogen was determined by the Kjeldahl-Gunning-Arnold method as outlined in Official Methods (*2*, *p. 8* [*24*]). The analytical data are presented in figures 3 to 8.

EXPERIMENTAL RESULTS

CHEMICAL COMPOSITION OF ROOTS

DRY MATTER

The percentage of dry matter in pecan roots fluctuated considerably during the course of the experiment (fig. 3). These fluctuations occurred at approximately the same time in roots of all trees but were less in roots of trees headed to stumps and first forks than in any of the others. The percentage of dry matter was apparently largely determined by the moisture supply in the soil and its absorption and storage in the roots, since in general the least dry matter was found in winter and spring when the soil moisture was relatively abundant. That the fluctuations were not due to a great extent to changes in amounts of the constituents of the dry matter is shown by the fact that starch increased at times when the percentage of dry matter was decreasing. The relatively high maximum concentration of starch and its wide fluctuation would have an appreciable effect on the dry-matter content, but not nearly so much as water absorption by the roots.

SUGARS

The reducing-sugar content of roots of the trees in all treatments was relatively low throughout the experiment, but after May 1932 roots of the stumps contained less than any others. At the end of the experiment there was little difference in the amount of reducing sugars in any of the other treatments (fig. 4).

The nonreducing sugars behaved in a manner similar to the starch. There was a larger amount and greater fluctuation in the roots of the trees least severely headed (fig. 4). The fluctuations of nonreducing sugars were, in general, somewhat parallel to those of starch after May 1932. In the roots of trees headed to stumps the nonreducing sugars were low throughout the experiment, while in those headed to first and second forks there was an increase beginning about the middle of 1933 and continuing to late fall. In general, the nonreducing-sugar content in roots of trees headed to third and fourth forks was lower than in roots of check trees until near the end of the experi-

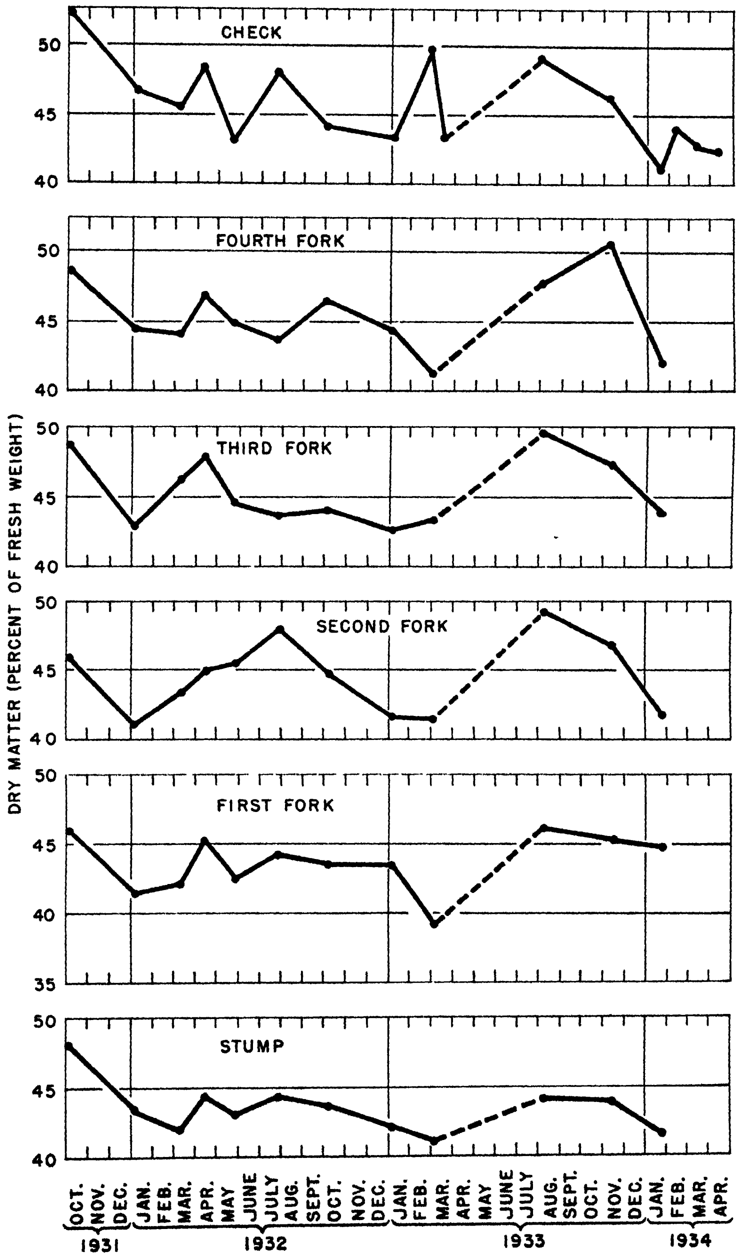

FIGURE 3.—Percentage of dry matter in roots of normal (check) trees and those headed to different degrees of severity in top working.

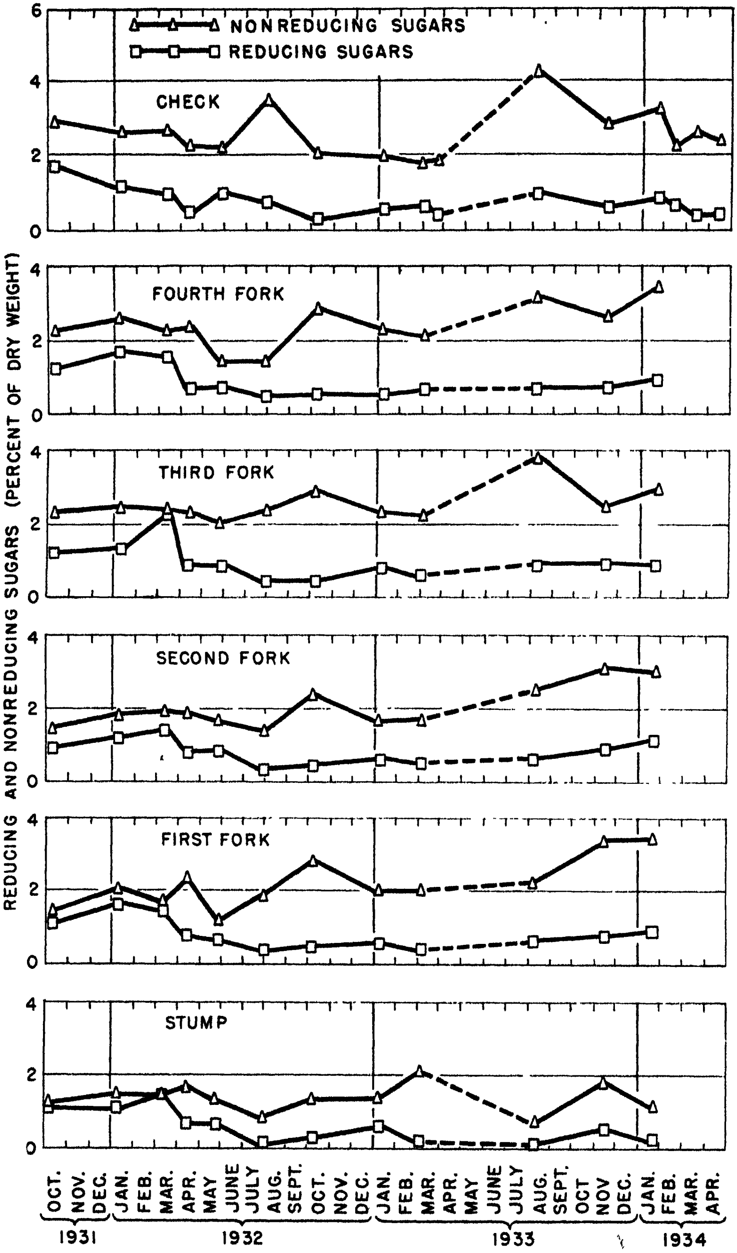

FIGURE 4.—Percentage of reducing and nonreducing sugars in roots of normal (check) trees and those headed to different degrees of severity in top working.

ment. There was also somewhat less fluctuation of the nonreducing sugars in roots of these trees than in the check trees. When the values for nonreducing sugars are calculated on a fresh-weight basis and then plotted, they form a curve that parallels somewhat the curve for dry matter (fig. 5). This shows that the concentration of these sugars varies to some extent with the variation in dry matter; however, calculations show that the change in the amount of dry matter was usually not proportional to the change in amount of nonreducing sugars. Therefore, a part of the variation in nonreducing sugars was due to actual increases or decreases of the total amount in the roots. The data are given for check trees only, since those for headed trees show similar variations.

STARCH

The data for starch analyses are given in figure 6. The starch content in the roots of check trees showed pronounced fluctuations that seemed to be more or less seasonal in character. The root samples were not taken at sufficiently frequent intervals to determine the exact seasonal trends, but the data indicate that in general starch increases from a minimum in summer to a maximum in autumn, and then decreases to a minimum in summer again. This is in line with what LeClerc du Sablon (*7*), Murneek (*9*), and Davis (*4*) have found in various deciduous trees.

In 1931 the starch in the roots of check trees did not reach a maximum in the autumn but continued to increase through the winter to a maximum in March. In each of the other two seasons the starch concentration attained a maximum in autumn and then decreased during the winter. The only known differences in the conditions during 1931 as compared with 1932 and 1933 are that the nut crop of 1931 was much smaller and the leaves remained on the trees later in the fall.

With the initiation of growth in the spring of 1932 there was a rapid disappearance of starch from the roots; the starch was probably used in growth and blossom development. From the last of May to October 5 there was a gradual increase in the starch. This increase is probably due to storage of the excess assimilated from photosynthesis by the leaves. Little vegetative growth was being made at this time, and the nut crop had not begun to fill; the leaves were full-grown and probably capable of maximum photosynthesis.

The starch decreased during the nut-filling period, which was completed about the middle of November, and continued to decrease until the following March. The nut crop was large and made a heavy demand for carbohydrates for oil formation in the kernels (*11*), and the starch reserve was probably used to supplement the carbohydrates produced by photosynthesis. Defoliation occurred shortly after the nuts were harvested, and therefore the starch reserve could not be built up again by photosynthetic activity. It will be noted that there was also a rapid decrease in starch after leaf fall in 1933, which seems to corroborate the view that there is usually a winter decrease of starch in roots of deciduous trees caused by its translocation, in the form of simpler carbohydrates, from the roots to the aerial portions of the trees.

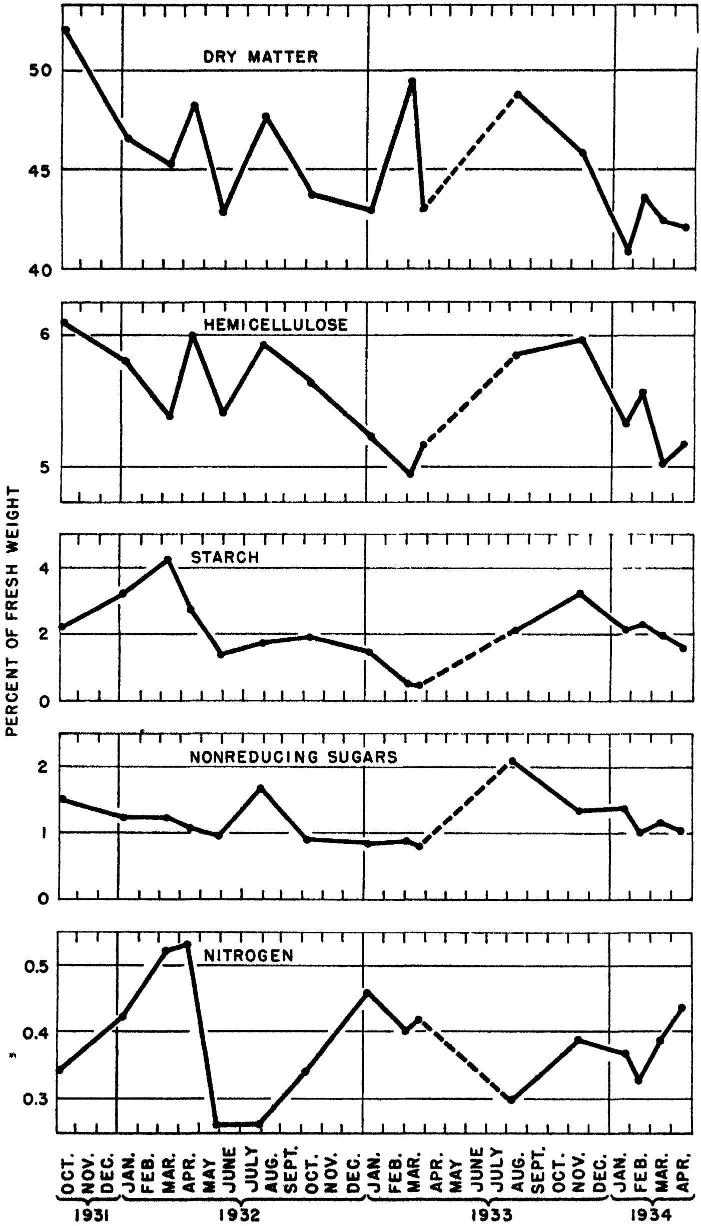

FIGURE 5.—Percentage of dry matter, hemicellulose, starch, nonreducing sugars, and nitrogen on a fresh-weight basis in roots of normal (check) trees. Note that the concentration of hemicellulose and nonreducing sugars varies to a considerable extent with the variation in dry matter.

From March to November 1933, when the nut crop was light and the conditions in late summer and fall were poor for vegetative growth, the starch content increased to a relatively high level and then decreased as in the season of 1932.

Although the data shown in figure 6 were calculated on a dry-weight basis, the same type of curve is obtained by plotting the amounts of starch in roots of the check trees calculated on the fresh-weight basis (fig. 5). A comparison of this curve with the one for dry matter indicates that the starch fluctuations are largely independent of dry-matter variations and to a great extent are caused by actual increases or decreases in the total amount of starch in the roots. In fact it would be impossible for the changes in amount of dry matter to cause the starch fluctuations in most cases because the starch content on a fresh-weight basis increases with decreases in dry matter. The reverse is also true, and the proportionate variations in the two constituents are not the same.

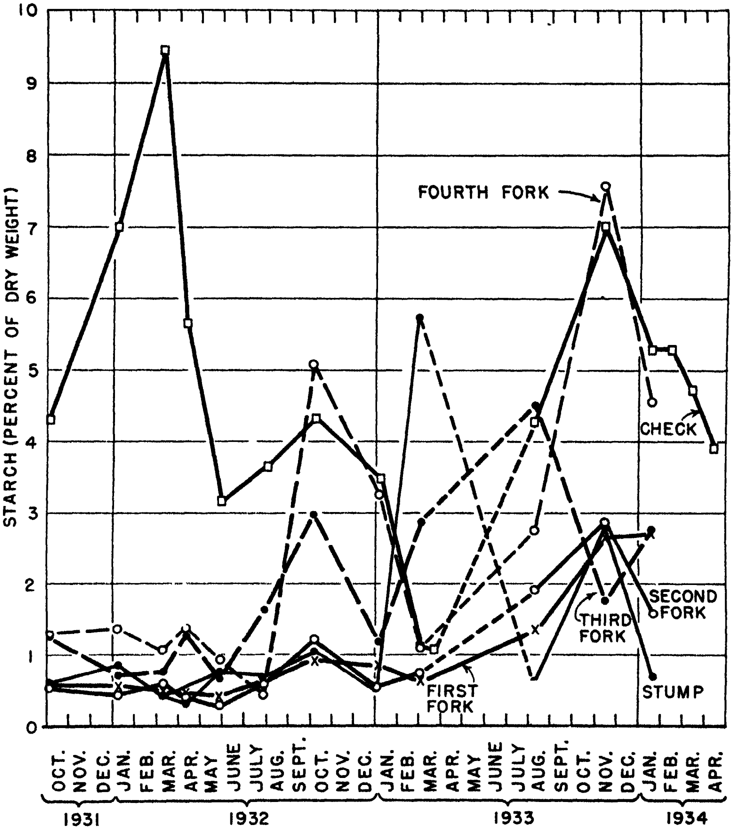

FIGURE 6.—Percentage of starch in roots of normal (check) trees and those headed to different degrees of severity in top working.

The starch content in the roots of all the cut-back trees was very low from October 1931 to the middle of the summer of 1932, and showed relatively little fluctuation regardless of the degree to which the trees were cut back. Subsequent to August 1932, however, there was a progressive increase in the amount of starch in the roots of trees under the third- and fourth-fork treatments. In the roots of trees headed to third forks the starch began to increase during the summer of 1932, and it fluctuated in the same manner as that in check trees but to a lesser degree. The starch content in roots of fourth-fork trees started to increase at the same time and during the latter part of the experiment was as high as that in roots of the check trees. Its fluctuation was also very similar to that in the roots of check trees, in both manner and degree, during this period.

The amount of starch and its fluctuations in roots of first- and second-fork trees were very similar throughout the experiment. There was no progressive increase in starch content until the summer of 1933, and at the end of the experiment it was still relatively low. The starch content of the roots of trees cut to stumps was low throughout the experiment, except for one abnormally high concentration on March 3, 1933. The reason for this large value is not known.

HEMICELLULOSE

The average amount of hemicellulose in roots of the trees under the different treatments was about 12 percent on a dry-weight basis and showed no consistent seasonal variations (fig. 7). However, on a fresh-weight basis the hemicellulose varied with the dry matter (fig. 5), indicating that the changes in the hemicellulose values are due largely to changes in dry matter rather than to changes in the total amount in the roots. Therefore, it is apparent that the hemicellulose was not utilized as a reserve carbohydrate to any appreciable extent.

NITROGEN

The amount of nitrogen in the roots of trees headed to stumps was, in general, somewhat larger than in the roots of the trees under any other treatment, but it showed practically no seasonal fluctuation (fig. 8). In roots of trees headed to first and second forks the nitrogen content showed somewhat greater fluctuations, which appeared to be seasonal except that there was a continued decrease in the amount of nitrogen after March 3, 1933, to the end of the experiment. In the roots of trees headed to third forks the amount of nitrogen was as low as or lower than that in roots of second-fork trees, but it showed more definite seasonal fluctuations. The nitrogen increased from October 1931 to April 12, 1932, after which it decreased to July 27. Then there was a gradual increase to March 3, 1933, followed by a decrease to August 9, and then an increase again to January 23, 1934.

The nitrogen content in roots of trees headed to fourth forks was a little higher as a rule than in roots of third-fork trees and showed greater seasonal fluctuations, which coincided with those in the trees under third-fork treatment. In roots of the check trees was found the greatest seasonal variation in nitrogen. The fluctuations occurred at the same time as those in the third- and fourth-fork trees but were wider over the period of the experiment.

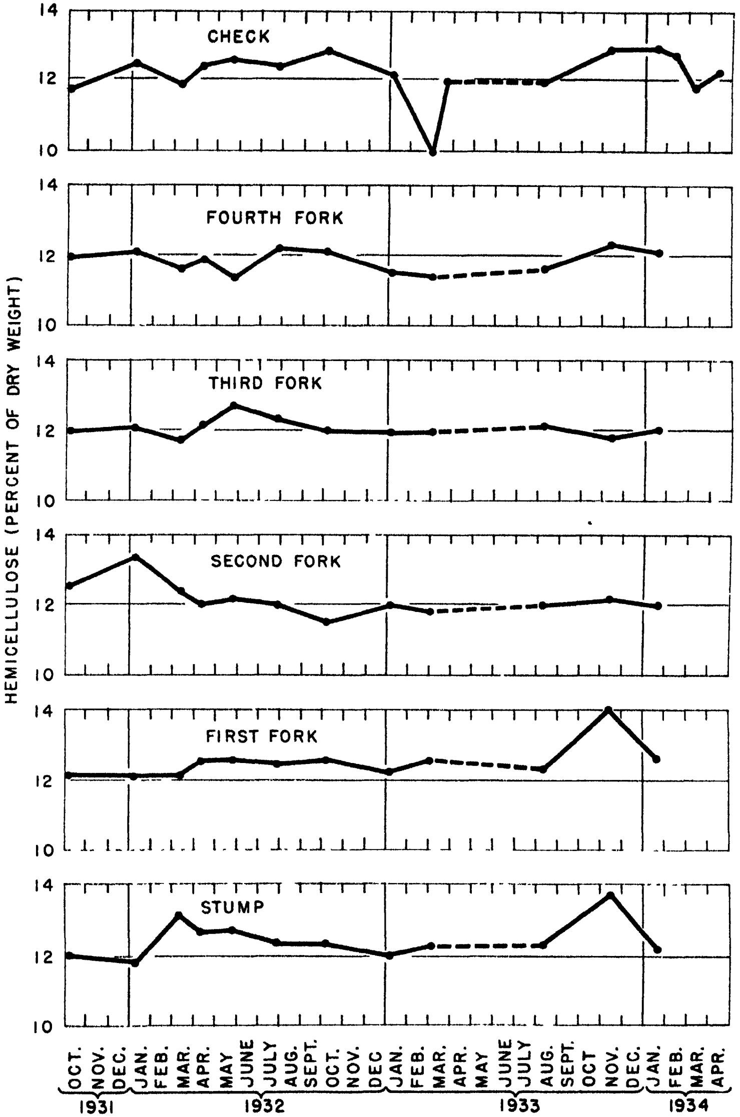

FIGURE 7.—Percentage of hemicellulose in roots of normal (check) trees and those headed to different degrees of severity in top working.

A comparison of the dry-matter and nitrogen curves in figure 5 shows that the nitrogen fluctuation was independent of the variations in dry-matter content. In general, nitrogen increased on both the fresh-weight and dry-weight bases as the dry matter decreased, and vice versa. It is probable that this was only coincidental, however, and that neither factor was the cause of the other.

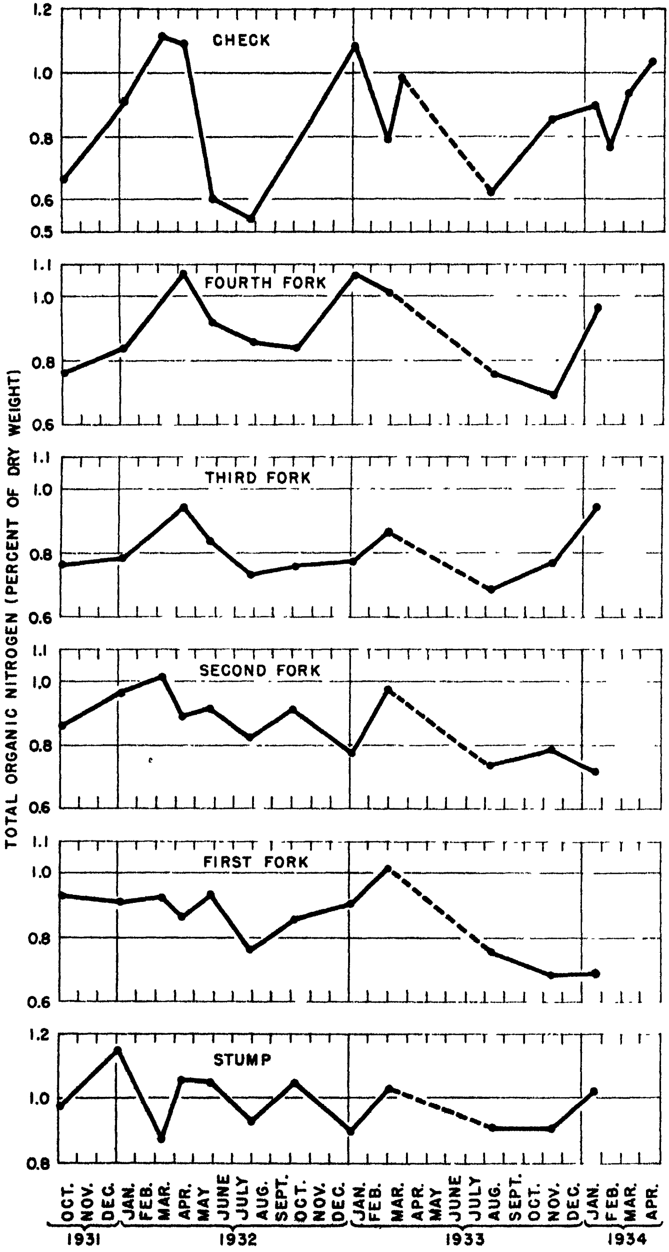

FIGURE 8.—Percentage of total organic nitrogen in roots of normal (check) trees and those headed to different degrees of severity in top working.

TREE GROWTH

Gain in Cross-Sectional Area

The following data show the average increase from March 1931 to December 1933 in cross-sectional areas of the trunks of normal (check) trees and trees headed to different degrees of severity in top working.

Treatment:	*Square inches*[1]
Normal (check) trees	22.60
Fourth-fork trees	5.61
Third-fork trees	6.34
Second-fork trees	3.98
First-fork trees	3.53

[1] Average increase in cross-sectional area of trunks

This increase is greater the less severe the heading treatment, except that the third-fork trees made somewhat more trunk growth than fourth-fork trees. The check trees show an increase in trunk growth more than three times as great as any of those cut back. No measurements are recorded for trees given the stump treatment, since most of these died to the ground before the end of the experiment.

Top Growth and Healing of Wounds

It is believed that the amount of top growth of cut-back trees is a much more accurate measure of their actual growth than the increase in cross-sectional area of the trunks. No measurements were made of the top growth of trees under the different treatments, but the relative

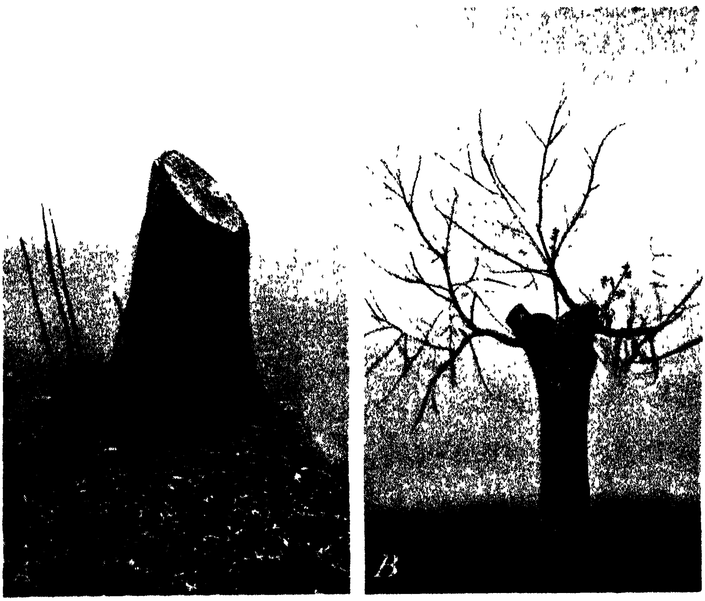

Figure 9.—Pecan trees cut back March 1931, headed to different degrees of severity. *A*, Headed to stump (photographed April 1932); grafts placed at the top were blown off by wind; stump dead to ground and sprouts coming from roots. *B*, Headed to first fork (photographed April 1934). Note that the large wounds have not started to heal.

amounts of this growth and the degree of healing of wounds are shown by photographs of typical trees near the end of the experiment (figs. 9 and 10).

All the large trees that were cut back to the stumps died to the ground, and sprouts came up from the roots (fig. 9, *A*). A few of the smaller stumps lived, but the wounds have made little progress in healing, and much difficulty has been experienced in preventing grafts and buds from being blown off by winds.

The first-fork trees have made relatively little top growth, and the large wounds have not begun to heal (fig. 9, *B*). Many of the new branches that grew on these trees were blown off by winds, since the unions with the large limbs were not strong.

Trees in the second-fork treatment made considerably more top growth than first-fork trees, but the wounds have made little progress in healing (fig. 10, *A*). Some of the new branches on these trees were also blown off by winds.

The top growth in third-fork trees was much greater than in second-fork trees, and the wounds are partially healed (fig. 10, *B*).

The fourth-fork trees made more top growth than any other cut-back trees, and the wounds are practically healed (fig. 10, *C*). The tops are now reestablished to such an extent that the trees can bear large crops of nuts. No loss of buds or grafts from windstorms occurred in these trees or in those cut back to third forks.

DISCUSSION

The data show certain definite differences in the composition of roots of check trees as compared with those of trees that were cut back in top working. These differences were nearly the same for all cut-back trees during the first season but tended to disappear as the tops were reestablished.

Starch and nitrogen were the largest variables of the constituents determined, and their fluctuations seemed to be more or less seasonal. The data for starch seem to indicate a summer minimum and a fall maximum, with a small winter decrease following leaf fall and a rapid decrease during spring growth and blossoming. In the fall of 1931, however, the starch in the roots of check trees increased from October until the following March, while in 1932 and 1933 it reached a maximum in the autumn and then decreased during the winter. The nut crop of 1931 was very small, the conditions were poor for vegetative growth, and the foliage remained on the trees until late in the fall. Under these conditions the excess carbohydrates produced by photosynthesis were probably translocated to the roots and stored as starch. There may also have been some weather conditions prevalent during the period that were favorable for the continued translocation of carbohydrates from the aerial portions of the trees to the roots, whereas in the other two seasons such conditions were absent. Murneek and Logan (*10*), in a study of autumnal migration of nitrogen and carbohydrates in the apple tree, concluded that since starch and possibly other more complex carbohydrates are hydrolyzed to sugars with the onset of cold weather, or vice versa, modifications induced by weather often are of greater amplitude than possible seasonal trends.

The starch in the roots of check trees reached its lowest concentration in the spring following the large nut crop of 1932. The starch

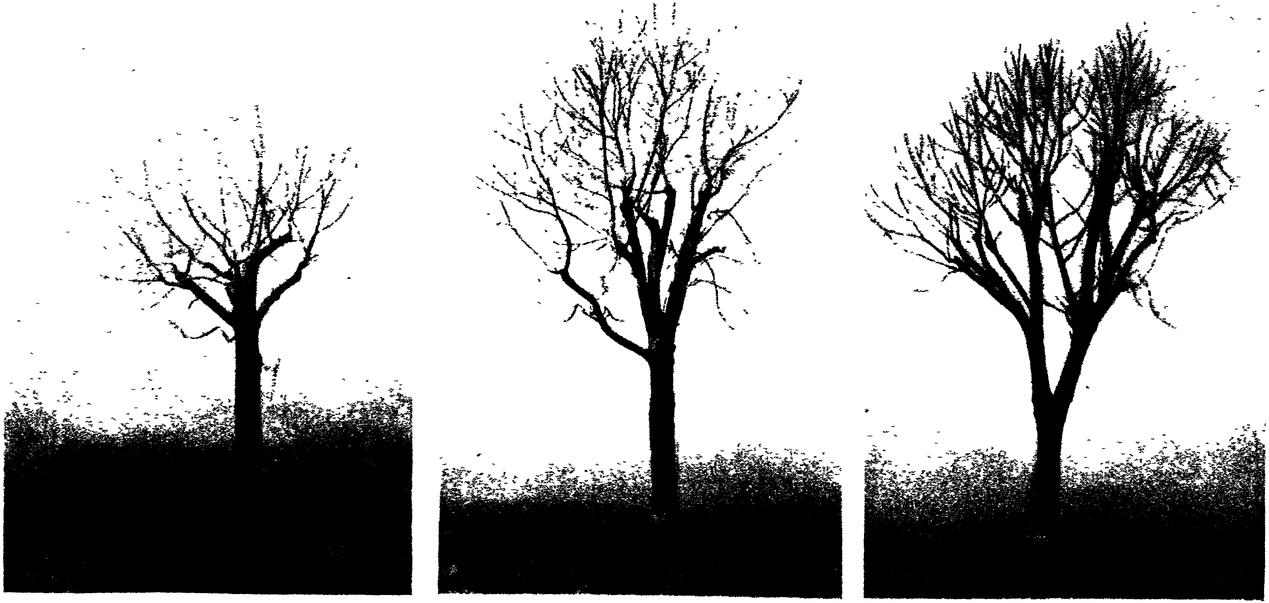

FIGURE 10.—Pecan trees cut back March 1931 (photographed April 1934), headed to different degrees of severity. *A*, Headed to second forks; large wounds have made little progress in healing. *B*, Headed to third forks; note that the wounds are relatively small and are partially healed. *C*, Headed to fourth forks; the wounds are small and practically healed; the tops are large enough to bear good crops of nuts.

decreased rapidly during the nut-filling period and, no doubt, was used in oil formation in the kernels. Thor and Smith (*11*) have shown that there is a heavy demand for carbohydrates during the period of oil formation in the pecan nut in the early fall, and that most of this carbohydrate material is translocated to the nut during this period. On the basis of microscopic studies, Finch and Van Horn (*5*) concluded that starch storage in fruit-bearing shoots of the pecan was initiated in early summer, reached a maximum shortly before nut filling began, and then decreased rapidly during this period. A comparable decrease did not occur in nonfruiting shoots. Davis (*4*) found that the starch content in the roots of nonbearing sugar-prune trees was much higher than in roots of bearing trees. He also observed a very rapid decrease of starch in the bark, spurs, and wood of bearing trees during the period of ripening of the fruit, while during the same period there was not a comparable decrease in nonbearing trees. With the beginning of growth in the spring, there was a decrease in starch content of both bearing and nonbearing trees, which attained a minimum at blossoming, but the minimum in the bearing trees was much lower than in those not bearing. This probably explains the very low concentration of starch in the spring of 1933 following the large nut crop of 1932. The starch content of the roots was depleted during the nut-filling period; and, since the foliage dropped soon after the nuts were harvested, the starch was not built up again by photosynthesis. Consequently, with the normal winter decrease of starch and the initiation of growth in the spring, the starch had reached a very low level on March 3. No analyses were made during the period of shoot growth and blossoming in the spring of 1933, but it is likely that the starch continued to decrease during this period since the data for 1932 and 1934 show decided decreases in the starch during similar periods.

The starch was relatively very low in the roots of all headed trees and showed little fluctuation during the first part of the experiment, but there was a progressive increase in its amount and fluctuation during the latter part of the experiment. Therefore, it seems evident that the quantity of starch is controlled largely by the growth and fruiting of the tree. In a study of the relation of leaf area and position to quality of fruit and to bud differentiation in apples, Haller and Magness (*6*) found an increase in blossom-bud formation and in size of fruit with an increase in leaf area on ringed branches, thus indicating that sufficient synthesis of food materials for blossom-bud differentiation and fruit development is obtained only with a relatively large leaf area. No doubt a similar condition prevails in the pecan tree, since the data seem to corroborate this view. During the first season after the trees were cut back the leaf area per tree was small under all treatments. The amount of leaf area was practically inversely proportional to the degree of severity of heading, but in no case was it adequate to synthesize sufficient carbohydrates to support growth, heal the wounds, and have much excess for storage in the roots. A considerable number of the leaves that grew on the new branches during the first season were removed when the top working was done in order to force out the buds and grafts. Thus there was actually less difference in the leaf area of trees in the different treatments during this period than if no leaves had been removed, and consequently there was very little difference in the amount and fluctuation of starch.

During the latter part of the experiment much of the leaf area of trees under the stump, first-fork, and second-fork treatments was lost because the branches that grew from buds or grafts were blown off by wind. The loss of these branches also necessitated rebudding the trees and removing still more leaf area to force the buds. This partly accounts for the low starch values in the trees under these treatments at the end of the experiment as compared with those in the check trees, or those under the third- and fourth-fork treatments. The trees headed to third and fourth forks bore more branches and leaves than the severely cut-back trees, and practically none were blown off by winds. Therefore, toward the latter part of the experiment the tops of these trees had been reestablished to such an extent that the amounts of starch and its fluctuation in the roots were very similar to those in the check trees.

The data indicate that the sugars in pecan roots are not storage but labile forms into which other more complex carbohydrates may be converted for use or translocation. The amount of reducing sugars was relatively low in the roots of trees under all treatments, but after May 1932 the roots of stumps contained less than any others. The concentration and fluctuation of nonreducing sugars were greater in the roots of check trees and those least severely headed, the fluctuations being somewhat parallel to that of starch during the latter part of the experiment. The larger amount of sugars in roots of the check trees and those not severely headed, as compared with those that were, no doubt is a direct result of the greater supply from the larger leaf area, and the wider fluctuations may be due to the usage during growth and fruiting activities.

The concentration of nitrogen in the pecan roots was about the same for check trees and those cut back except that it was slightly higher in the roots of stumps. However, the extent of the seasonal fluctuations of the nitrogen was somewhat inversely proportional to the degree of severity of heading.

The nitrogen increased during the fall and winter and decreased during the spring, which indicates that it is used mainly in growth and blossom development. During late summer and fall when the vegetative growth has practically ceased it is assumed that the nitrogen continues to be absorbed and is stored. Weinberger and Cullinan (*12*) found that nitrogen was absorbed in large amounts by the roots of peach trees in late fall. They state (*12, p. 67*): "The roots of fertilized trees, however, practically doubled in nitrogen content in the same period [October 19 to December 22], compared to only a 15 per cent increase in check roots." Aldrich (*1*) obtained similar results with fall and winter applications of nitrogenous fertilizers to apple trees. Crane (*3*) has shown that fall applications of nitrogenous fertilizer are about as effective in increasing the growth and yield of pecan trees as are similar applications made in the spring. Loomis (*8*) presents data which indicate that nitrogen is rapidly synthesized to organic forms upon absorption by the roots. His data indicate that nitrogen is normally translocated in organic forms through the phloem, and that the process of digestion and translocation may be very rapid. In the bark of 3-year-old poplar trees one-half the nitrogen was digested and moved from the trunks in the first 4 weeks of the growing season. Murneek and Logan (*10*) found that there was an autumnal migration of nitrogen from the leaves to the stems prior to leaf fall in the apple. A part of the increase in nitrogen in the pecan roots occurring in the fall may have been due to the nitrogen that was translocated from the leaves prior to their abscission.

In the spring there is a large demand for nitrogen for the production of vegetative growth and blossoms, which probably cannot be met by absorption at that time. The nitrogen stored in the roots is probably translocated to other portions of the tree for these purposes, and the root content is thereby decreased. The fact that there was greater seasonal fluctuation in the amount of nitrogen in roots of the check trees and in those with less severe heading, where more growth and fruiting occurred, than in trees severely cut back, where little growth and fruiting took place, is further evidence that the explanation given above is valid.

According to the data the actual amount of nitrogen present at any one time could not be used as a measure of the physiological activity of the tree, since in the roots of trees headed to stumps the nitrogen present was generally as high as or higher than in the check trees. There was so little growth activity in the stumps that the root system was able to absorb nitrogen rapidly enough to supply this demand, and consequently the nitrogen remained nearly constant.

SUMMARY AND CONCLUSIONS

Pecan trees of an average diameter of about 12 inches were headed back in March 1931 to stumps, first forks, second forks, third forks, and fourth forks prior to being top-worked to improved varieties. Analyses of roots of these trees and of similar untreated ones were made at intervals from October 1931 to January 1934 for dry matter, reducing and nonreducing sugars, starch, hemicellulose, and organic nitrogen.

The dry-matter content of roots of pecan trees was higher in the summer and autumn than in the winter and spring, and apparently these fluctuations were controlled largely by transpiration and the moisture supply in the soil. The dry matter fluctuated less in roots of trees headed to stumps and first forks than in roots of check trees or those headed to second, third, and fourth forks.

Although the root samples were not taken at sufficiently frequent intervals to determine exact trends, the data indicate that starch increases from a minimum in summer to a maximum in fall or early winter, and then decreases again to a minimum in summer; also, that the fall maximum may be prevented in a heavy crop year because of the demand for carbohydrates for oil formation in the kernels of the nuts during filling.

The amount and fluctuation of starch in pecan roots appear to be largely dependent upon the amount of leaf area and the growth and fruiting of the trees. During the first year after the trees were cut back, the leaf area on all headed trees was relatively small, and the starch content was low and showed no seasonal variation. After this time, however, the amount of foliage showed an inverse relationship roughly proportional to the severity of heading back, and the amount of starch and its fluctuation were about proportional to the leaf area.

In the roots of check trees the starch content fluctuated widely, and its concentration was relatively high except in the winter and spring following the large nut crop of 1932.

The reducing-sugar content in the roots of all the trees was low and about equal throughout the experiment except in the stumps, where it was lowest during the last half of the experiment. The nonreducing sugars behaved in a manner similar to starch. The amounts were higher and there was more fluctuation as the leaf area per tree was increased. In general, the seasonal fluctuations of nonreducing sugars were nearly parallel to the starch variations but apparently these fluctuations were partly due to changes in the dry-matter content of the roots. The indications are that sugars in pecan roots are not storage but labile forms into which other more complex carbohydrates are converted for use or translocation.

There were only moderate fluctuations in the hemicellulose content in the roots of trees under the different treatments, and these variations were caused largely by changes in the dry-matter content of the roots.

The amount of nitrogen was somewhat higher in the roots of trees headed to stumps and fluctuated less than in those under any other treatment. In the roots of the check and of the third- and fourth-fork trees the amount of nitrogen fluctuated widely, increasing from late summer to early spring and then decreasing again, indicating that its main function is in growth and blossoming. In roots of first- and second-fork trees this same seasonal fluctuation occurred during the first 2 years of the experiment, but during the last year the nitrogen continued to decrease from March to January. The seasonal fluctuations in nitrogen concentration were independent of changes in dry-matter content of the roots.

For comparing the growth made by pecan trees during the first few years after they had been cut back in top working, the amount of top growth appears to be a better measure than the increase in the cross-sectional area of the trunk.

The rate of healing of wounds occasioned by the heading of trees was approximately proportional to the amount of leaf area per tree.

The data, together with observations made on the rate and facility of healing of wounds in the different treatments, indicate that, in heading pecan trees preparatory to top working, cuts more than 4 inches in diameter should be avoided. Larger cuts are hard to heal and, in heading trees severely enough to make wounds larger than 4 inches in diameter, the potential leaf area is automatically so reduced that it will probably be inadequate to synthesize sufficient foods for the normal recovery of the tree.

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STUDIES ON THE NATURE OF BROWN ROOT ROT OF TOBACCO AND OTHER PLANTS[1]

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INTRODUCTION

Despite the highly specialized adaptation of roots to soil, it is surprising that decay of these organs is not more frequent, considering the varied environment to which they are exposed under intensive cropping. Normally it is only when a specific parasite is concerned that extensive root injury occurs on plants. When, therefore, a root necrosis develops frequently on a crop without any obvious parasite or unfavorable environment being present, it offers a problem of special interest to pathology. The brown root rot disease of tobacco (*Nicotiana tabacum* L.) appears to be a malady of this type, which has now been recognized for 20 years, particularly in the more northerly tobacco-growing States. The disease may affect a wide variety of plants, but evidently no serious economic losses from this cause have been reported to occur except on tobacco. Paradoxical as it may seem, the disease is favored by certain apparently nonsusceptible crops when used as preceding crops in rotations with tobacco, whereas the continuous culture of certain susceptible crops results in the gradual elimination of the causal agent of brown root rot from the soil. This conclusion, based upon many field experiments and observations over a 20-year period, together with repeated failure to isolate an organism that produces infection, has naturally led to reasonable doubt that a typical parasite is concerned.

It is not the object of the present paper to offer a new hypothesis to explain the disease, but rather to bring together the results of certain investigations undertaken for the purpose of securing further information regarding the nature of the causal agent. These relate chiefly to the general behavior and properties of the causal agent and the pathological histology of the disease. A brief summary of the results presented in this paper has been previously published (*9*)[3].

LITERATURE REVIEW

It is not deemed necessary in the present connection to consider the various ramifications of the problem in the literature relating to "soil toxins" and the injurious effect of preceding crops. The former has recently been reviewed by Loehwing (*13*), and the latter subject was treated by Garner et al. (*7*) in some detail in 1925. Toxic action on plant growth may be induced in artificial culture, and crop retardation

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[2] The writer is indebted to C. V. Kightlinger, Division of Tobacco and Plant Nutrition, Bureau of Plant Industry, U. S. Department of Agriculture, for help in selecting the soils used and for shipping the soils for experimental work to the Wisconsin laboratory.

[3] Italic numbers in parentheses refer to Literature Cited, p. 862.

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may develop as a result of cropping systems in the total absence of root decay; the problems there involved are probably quite different from the brown root rot problem. Brown root rot, as far as can be judged by its behavior and symptoms, is a specific disease, although in the absence of a known causative agent it may not always be readily or definitely determined as such. The first detailed description of the malady was made in 1926 from this laboratory (*11*). In addition to the description of the disease, the failure to secure infection from the organisms isolated from diseased roots was emphasized. The destruction of the causal agent by heat and formalin sterilization as well as by desiccation of the soil was reported. Field experiments in the Connecticut Valley showed the strikingly injurious effect of certain preceding crops on succeeding crops of tobacco. The retarded growth was definitely associated with the presence of the brown root rot disease. Since 1926 continued field experiments of a similar type have been conducted in the Connecticut Valley and in Wisconsin, and these have yielded very similar results; consequently the data have not been published in detail (*10*, *15*).

One line of investigation was developed further, however, by Thomas (*17*), who attacked the problem from the point of view of the chemistry of the soil. Thomas secured high degrees of depression of plant growth, accompanied by root discolorations resembling brown root rot, as a result of adding relatively pure forms of cellulose (filter paper) to the soil. The abundance of energy material furnished by the cellulose stimulated the growth of fungi to such an extent that a shortage of available nitrogen followed, resulting in depressed plant growth. More specifically, however, it was suggested that these normally saprophytic soil fungi, in their further search for nitrogen, invaded the root tissues of plants sufficiently to cause the necrosis known as brown root rot. This theory was later expanded by Mathews, Reneger, and Thomas (*14*), who attributed the disease to the specific action of *Rhizoctonia bataticola* under similarly favorable soil-environment conditions.

In the meantime other workers in the Connecticut Valley (*1*, *4*, *5*, *6*, *12*, *16*) have leaned more directly toward the theory that a toxin or an unbalanced mineral nutrition may be the cause of brown root rot. Infusions of decomposing plant tissues, especially of timothy, for example, were found to depress plant growth as well as to cause discolorations and necroses of the roots resembling brown root rot. The decomposition of the plant tissues and the constituents of the infusions are naturally a result of microbial action and, although the toxicity of such infusions has been demonstrated, the results do not conclusively show that similar circumstances are responsible for the field conditions known as typical brown root rot. Nevertheless, until plant roots are treated with such toxins under aseptic conditions, some doubt may still exist as to the chemical explanation of the resulting necrosis.

That brown root rot is due to the presence rather than to the absence of some factor is indicated by its destruction by heat or antiseptics. It does not seem likely that the disease may be attributable to the lack of normal plant-food constituents in the soil. So far as known, no one has intensively studied the possible remote relation of a deficiency of the rarer elements to the disease.

MATERIALS AND METHODS

The soils used in these investigations were largely secured from known brown root rot fields in the Connecticut Valley, especially in the vicinity of Whately, Mass. Soils from other sections have been used for the laboratory work but in recent years have not given such heavy amounts of disease as have those from Massachusetts. The soils were stored in covered galvanized-iron cans or otherwise protected from drying out. The same lot of soil could thus be used over a period of at least 1 year without appreciable change in the amount of brown root rot present.

The experiments were largely performed during the winter months in the laboratory and greenhouse of the Wisconsin Agricultural Experiment Station. The tests for amounts of disease were made in 4-inch pots holding 500 to 600 gm. of soil, the trials usually being run in duplicate but repeated several times. At the end of about 4 weeks the roots were removed from the pots, washed free from soil, and floated out in water against a white background in order to estimate more readily the amount of disease present. Various means of recording the amount of disease on the roots have been tried, but the simple method of indicating different degrees of infection with plus signs appears to be quite satisfactory for the present purposes. The amount of disease present is, of course, generally expressed in the relative plant growth, but, owing to the erratic behavior of this character, it was not found to be reliable as a measure of the amount of disease. Tomatoes were most frequently used as test plants, thereby avoiding any danger of confusion with black root rot (*Thielaviopsis basicola* (B. and Br.) Zopf.), which is common in tobacco soils but to which tomatoes are immune.

Special methods or techniques are briefly referred to in this paper under the respective experiments where they were used.

EXPERIMENTAL RESULTS

BEHAVIOR OF THE CAUSAL AGENT

Brown root rot possesses many characteristics of a parasitic disease, although it shows little or no indication of being of a very infectious or rapidly developing type. That is, the time required for "infection" is relatively long, the spread of the disease through the tissue is rather slow, and the causal agent must be comparatively concentrated to produce much injury. Normally it requires 3 to 4 weeks to secure good symptoms (fig. 1) from brown root rot soil in greenhouse trials, and it is often difficult to ascertain when the first development of a given lesion was initiated.

The time required for the first signs of the disease was approximately arrived at by allowing the roots of tobacco and tomato plants to grow through the hole in the bottom of clay pots into water in small glazed crocks. When the protruding healthy white roots were sufficiently long, they were transferred to other crocks containing 1 to 2 inches of a heavy water suspension of brown root rot soil. At intervals individual plants were removed without appreciable disturbance of the individual roots and the soil was rinsed off the roots, which were again placed in pure water. In this manner it was found that approximately 3 days' exposure was required to secure the first minute signs of the disease and that little or no further spread of the individual lesions occurred outside of the "infested" soil.

The relative concentration of the agent required to produce symptoms was determined by diluting the diseased soil with the same soil previously sterilized by heat (table 1). Depending upon the amount of brown root rot in the original soil, it required approximately 5 to 20 percent of infested soil to secure traces of brown root rot. At higher concentrations the amount of disease was roughly in direct proportion to the percentage of infested soil (fig. 2).

If diseased plants are transferred to disease-free soil they recover and new roots develop showing no further appreciable symptoms of brown root rot. Similarly, a change of weather conditions, which may either reduce the amount of the causal agent in the soil or check the rate of development of the disease (*11*) or both, often results in marked recovery in the same soil during the growing season. Young roots and young plants are most severely damaged by the disease. The lesions may be quite superficial on old roots and, as the plants grow older, new roots are likely to form faster than the disease progresses, with the result that recovery of plant growth may proceed rapidly once the balance has been shifted in favor of the host.

FIGURE 1.—Roots of tomato plants showing light-brown lesions normally characteristic of brown root rot. In severe cases of the disease the root system may entirely decay, with the result that the plants make little or no growth.

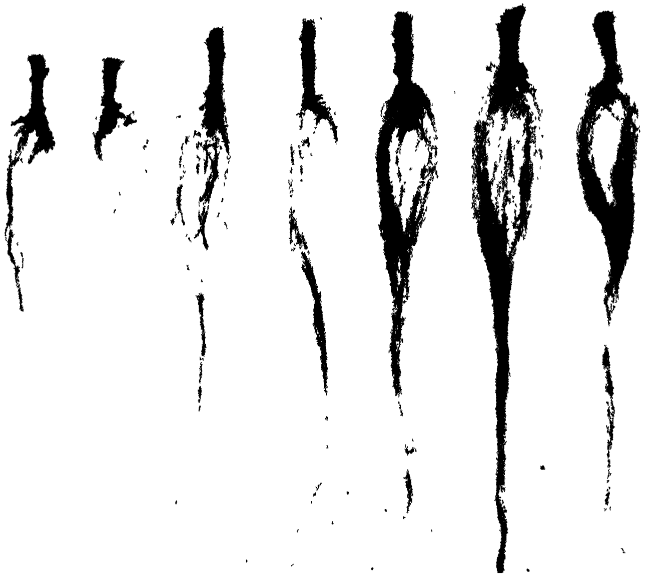

FIGURE 2.—Roots of tobacco plants showing comparative results in "infested" and disease-free soils and in mixtures in various proportions. If brown root rot soil is diluted with disease-free soil, the amount of disease in the roots is roughly in direct proportion to the percentage of the former soil in the mixture. From left to right, roots grown in 100, 80, 40, 20, 10, 5, and 0 percent of brown root rot soil.

TABLE 1.—*Influence of diluting 2 different brown root rot soils with sterilized soil on amount of disease developing upon roots of tobacco and tomato*

Brown root rot soil in mixture with sterilized soil (percent)	Amount [1] of brown root rot on—				Brown root rot soil in mixture with sterilized soil (percent)	Amount [1] of brown root rot on—			
	Tobacco plants grown in—		Tomato plants grown in—			Tobacco plants grown in—		Tomato plants grown in—	
	Soil 1	Soil 2	Soil 1	Soil 2		Soil 1	Soil 2	Soil 1	Soil 2
0	0	0	0	0	40	+++	+++	++	+
5	+	+	+	0	80	++++	++++	+++	++
10	++	+	+	0	100	++++	++++	+++	+++
20	++	++	+	+					

[1] 0=no disease; +=trace; ++=light; +++=moderate; ++++=heavy

Previous results have shown that the brown root rot agent cannot be extracted from the soil by leaching with water and that it is apparently not present in even small amounts in filtered water extracts of the soil (*11*). These observations have been repeatedly corroborated. It has also been shown that fresh diseased roots will reproduce the disease when introduced into the soil (table 2). If such fresh diseased roots are ground up and the juice is extracted, filtered through filter paper, and added to the soil, no disease is produced, although the pulp from which the extraction is made yields typical symptoms. Since the causal agent has not been secured in solution or suspension, it cannot be shown to be of any definite particle size. If the agent is of the nature of a toxin, it is possible that it might be dissolved by certain noninjurious chemicals, such as ether or acetone, and subsequently precipitated with other agents; but this has not yet been attempted.

Table 2.—*Effect of adding to disease-free soil brown root rot tomato roots treated in different ways*

Treatment of brown root rot roots added to soil	Amount of material added to 500 g of soil	Amount[1] of brown root rot on plants grown in—	
		Test 1	Test 2
Fresh roots..........grams..	18	++++	++++
Roots air-dried 24 hours..........do..	18	+	+
Roots sterilized..........do..	18	0	0
Fresh roots..........do...	25	++++	++++
Roots air-dried 24 hours..........do....	25	0	0
Extract from fresh roots..........cubic centimeters	20	0	0
Extracted pulp from fresh roots..........grams .	10	++++	++++
Healthy fresh tomato roots (control)..........do...	25	0	0

[1] 0=no disease, +=trace; ++++=heavy.

Since the agent is not soluble in water, further efforts were made to trace its distribution in the soil by means of physical fractionation of the soil with water. Brown root rot soil was shaken up repeatedly in large cylinders and allowed to settle out for definite periods of time. The soil used was very sandy so that about 95 percent settled out in 1 minute or less, and consequently considerable quantities had to be used to secure sufficient amounts of the silt and clay fractions for the test.

These preliminary trials showed that it was apparently impossible to wash the sand free from brown root rot with considerable quantities of water, since the sand fraction yielded quite as marked disease symptoms as the original soil. Approximately 1 part by weight of the soil fraction sedimenting between 1 and 24 hours from a 15-inch column mixed with 16 parts of sterile sand yielded nearly as much disease as did the control, showing that the agent was more concentrated in this silt portion than in the sand fraction. The finest clay or colloidal suspensions behaved like water extracts.

All the evidence goes to show that the brown root rot principle originates from or in the organic matter added to the soil through preceding crops; consequently, it is believed to be in highest concentration in such material or in its decomposition products. That organic matter carries the causal agent directly has been shown through the addition of the natural vegetable matter of brown root rot soils to disease-free soil, as well as by the use of diseased roots from brown root rot soil.

EFFECT OF DIFFERENT TEMPERATURES ON THE CAUSAL AGENT

Sterilizing brown root rot soil for a short period in the autoclave or in a steamer at 100° C. destroyed the disease-producing agent.

This behavior suggested that an organism capable of producing the disease was killed or that a toxic substance already present in the soil was destroyed. In seeking further information on this point, it was logical to determine more closely the actual temperature required to eliminate the causal agent.

A considerable quantity of soil was required to grow the necessary plants to determine the result of treatments, and consequently it was not possible to use short exposures to heat with a minimum quantity of soil. Beyond the commonly used 10-minute period of exposure for the thermal inactivation point, it was assumed that long exposures at low temperatures would yield more reliable results than short exposures, on account of the time required to heat soil to a uniform and definite temperature. With 1.2 kg. of moist soil spread out in a layer not more than 1 inch thick in a covered pan, the time of exposure in constant-temperature chambers was usually 24 to 48 hours. No appreciable drying of the soil took place under these conditions. The results presented in table 3 are sufficient to show that temperatures as low as 41° to 44° C., maintained for 24 hours, are very injurious to the brown root rot agent. At a temperature of 45° for 24 hours or 48° for 10 to 12 hours, the disease-producing agent is entirely eliminated. It was also found that a temperature of 45°, maintained for 24 hours, destroyed brown root rot in moist diseased roots subsequently used for inoculation in comparison with unheated diseased roots.

TABLE 3.—*Effect of heat on the causal agent of brown root rot, as determined by exposure of the soil to different temperatures for various periods*

Temperature (° C.)	Time of exposure	Amount [1] of brown root rot in—			
		Soil 1	Soil 2	Soil 3	Soil 4
	Hours				
48	48	0	0	-----	-----
45	48	-----	-----	0	0
44	48	0	0	-----	-----
41	48	0	0	-----	-----
48	24	0	0	-----	-----
45	24	-----	-----	0	0
44	24	+	0	0	+
43	24	-----	-----	+	+
41	24	++	++	-----	-----
48	10-12	-----	-----	0	0
Unheated controls	-----	++++	++++	++++	++++
100	1/3	0	0	0	0

[1] 0=no disease; +=trace; ++=light; ++++=heavy.

The maximum critical temperature for the growth of many microorganisms such as may be found in the soil is usually about 40° to 42° C., although most organisms are not killed by long exposures to temperatures of 45°. In experiments in which the same equipment was used as for the soil trials, *Radiobacter*, *Actinomyces scabies* (Thax.) Gues. and *Rhizoctonia bataticola* (Taub.) Butler all withstood 45° in culture for periods of more than 48 hours. These results appear to be especially significant in view of the report (*14*) that *R. bataticola* is associated with brown root rot. It is also worthy of note that the surface soil temperatures in the field may often reach 40° to 45° C. (104°–113° F.) for short periods during the day and that it is therefore likely that summer heat accounts for the disappearance of some of the brown root rot disease from the soil under field conditions.

Experiments on the effect of freezing on brown root rot were made by placing 1.2-kg lots of soil in covered refrigerator pans and exposing these in refrigerator chambers at different constant temperatures as well as out of doors at the variable freezing temperatures during midwinter. The time of exposure to these conditions ranged from 4 to 60 days. It became obvious at once that temperatures sufficiently low were capable of destroying the brown root rot agent in the soil. The critical temperature and time of exposure have not been accurately determined. It must suffice to conclude that freezing at temperatures of −5° C. (23° F.) for as long as 21 days is not necessarily injurious to the causal agent but that temperatures of −25° C. (−13° F.) for 10 days may completely destroy brown root rot (table 4). Exposure to the variable winter temperature of Wisconsin for a period of about 1 month (mean, 20° F.) was also effective in eliminating the agent from soil exposed to the weather in covered containers. In one trial it was found that a temperature of approximately −5° C. for 8 days effectively destroyed the agent in diseased moist roots when these roots were used as soil inoculum in comparison with unfrozen roots from the same lot. Again, it is obvious that this reaction of the brown root rot agent to freezing is not in accord with what may be expected of a typical plant parasite. It is also apparent that the low prevailing temperatures of the winter months in the Northern States may destroy a large part of the brown root rot in the surface layers of soil, especially if the land is not protected by snow or other material.

TABLE 4.—*Effect of freezing on the causal agent of brown root rot, as determined by prolonged exposure of the soil and moist diseased roots to various low temperatures*

Treatment	Time of exposure	Amount[1] of brown root rot in —			
		Soil 1	Soil 2	Soil 3	Soil 4
Soil exposed to —	*Days*				
−5° C. (23° F.)	21	++++	++++	--------	--------
−25° C. (−13° F.)	26	0	0	--------	--------
Control (soil not frozen)	--------	++++	++++	--------	--------
Soil exposed to —					
−25° C.	33	--------	--------	0	0
−25° C.	10	--------	--------	0	0
−25° C.	4	--------	--------	+	++
Outdoor temperature (20° F., mean)	33	--------	--------	0	0
Control (soil not frozen)	--------	--------	--------	++++	++++
Moist diseased roots[2] exposed to −5° C.	8	--------	--------	0	0
Control (moist diseased roots)	--------	--------	--------	++++	++++

[1] 0=no disease, +=trace, ++=light; ++++=heavy.
[2] Frozen before being added to sterilized soil.

EFFECT OF DRYING ON THE CAUSAL AGENT

It was shown in earlier studies on brown root rot (*11*) that air-drying the soil effectively eliminates the disease. This result has since been repeatedly corroborated. The method of drying used in the first experiments consisted simply in spreading the soil out in a thin layer in a dry room or out of doors on a clear day. In more recent tests the rate of drying has been more carefully regulated under controlled environmental conditions or in a partly covered container.

The data secured show quite conclusively that quick air-drying in as short a period as 5 hours is quite as effective as much slower drying. Very gradual air-drying of the soil over a period of several days or weeks, on the other hand, is far less destructive of brown root rot (table 5). In one instance, soil was maintained in a weighed covered pan for 120 days at 32° C. After 90 days, the soil lost no further weight, being completely air-dry. This slowly dried soil, when planted to tomatoes, yielded comparatively good symptoms of brown

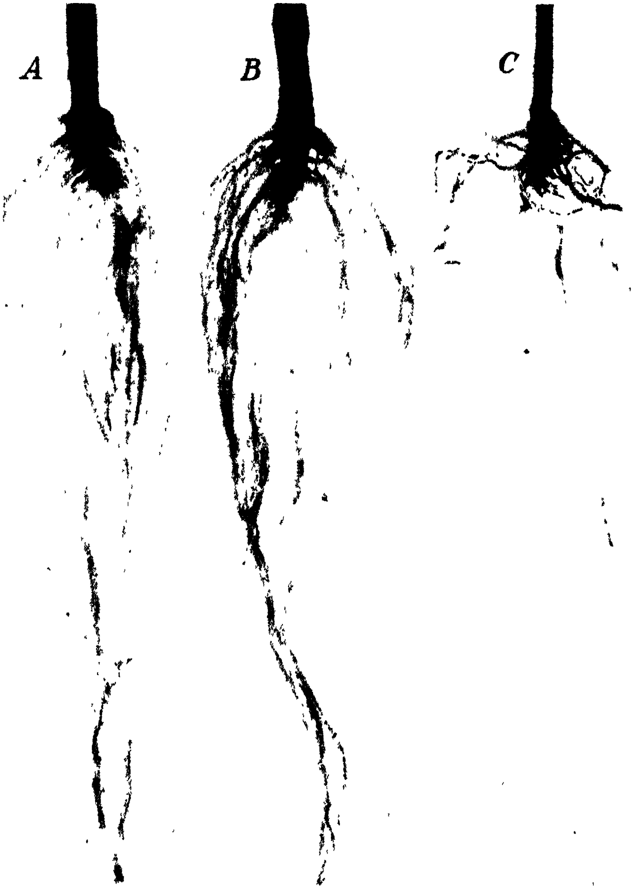

FIGURE 3.—Roots of tomato plants grown in soil to which roots affected with brown root rot have been added: *A*, Roots from soil containing sterilized diseased roots; *B*, roots from soil containing dried diseased roots; *C*, roots from soil containing fresh diseased roots. *A* and *B* failed to develop disease, whereas *C* became diseased.

root rot. No reliable explanation can be offered at present for this behavior.

Little is known about possible related phenomena, but the similarity of this behavior to the inactivation of the virus of ordinary tobacco mosaic in the soil by desiccation (*8*) is interesting and suggests that the disease-producing agent of brown root rot may be adsorbed by the drying soil. This explanation is hardly tenable, however, since desiccation is effective in destroying the active principle of brown root rot in the plant tissues as well (fig. 3), which is not true of the mosaic virus.

It is believed that the drying of the soil and possibly the rate at which drying takes place are of considerable significance in relation to the epidemiology of the disease. In the absence of a more definite means of diagnosis, air-drying may serve as a valuable method of identifying the typical brown root rot in doubtful cases.

TABLE 5.—*Effect on the causal agent of brown root rot of drying soil at different rates at 32° C.*

[Rate of drying regulated by covers on pans]

Time of drying (days)	Total loss of water from—		Amount [1] of brown root rot in—	
	Soil 1	Soil 2	Soil 1	Soil 2
	Percent	*Percent*		
2	15.0	12.4	+	0
8	12.7	10.8	++	++
28	14.8	12.3	++++	+++
None (control)	0	0	++++	++++

[1] 0=no disease, +=trace, ++=light, +++=moderate; ++++=heavy

EFFECT OF AERATION AND LIGHT ON THE CAUSAL AGENT

The exposure of brown root rot soil to drying by ordinary means also exposes the soil to greater aeration, a factor that may conceivably influence the causal agent through oxidation. In the earlier experiments, some attempts were made to aerate the soil for several days, without drying, by spreading it in a thin layer in a moist atmosphere (*11*). A later modification of this method consisted of the use of covered and open containers, with daily stirring for several weeks, and the maintenance of moisture content daily by weight. Results from this type of experiment are shown in table 6. Aeration by these methods had little or no effect on the amount of brown root rot.

Complete drying of considerable quantities of soil without aeration, or thorough aeration without drying at room temperatures, is not easily accomplished even with special equipment developed for the purpose. The first equipment used consisted of a slowly rotating glass cylinder (4 by 18 inches) for continuous stirring, through which was constantly passed an atmosphere regulated as to moisture and oxygen content (fig. 4). Some difficulties were encountered because of moist soil adhering to the walls of the revolving glass cylinder, but again it was found that the presence of pure oxygen for a week or its complete absence in continuously agitated soil had no effect on the brown root rot disease. More satisfactory results with less mechanical difficulties were later secured by the use of large side-tube desiccators to permit the change of atmosphere at intervals only. The drying agent used was calcium chloride. Nitrogen, oxygen, and air were introduced and replaced as desired. Other modifications permitted occasional stirring of the soil without disturbing the existing atmosphere in the container. In this manner, about 400 gm. of moist soil could usually be dried in 7 days in the presence or absence of air or pure oxygen.

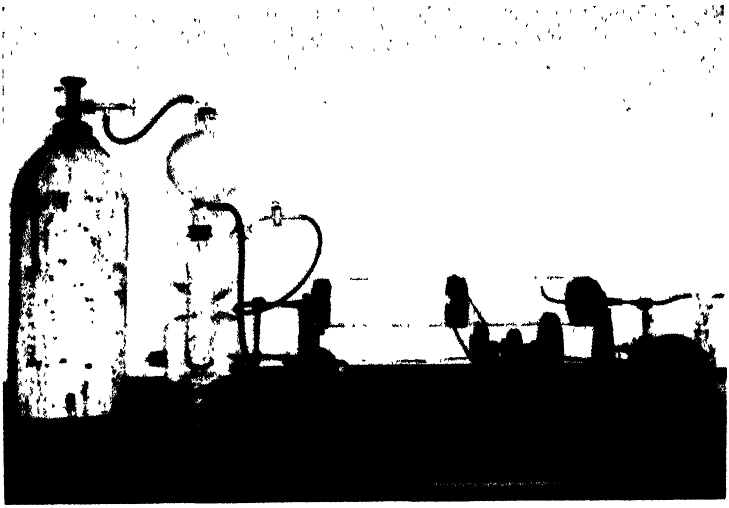

FIGURE 4.—Special equipment used for aerating brown root rot soil without drying, or vice versa. The soil is exposed in the slowly rotating glass cylinder for 7 more days to atmosphere regulated as desired in oxygen and moisture content.

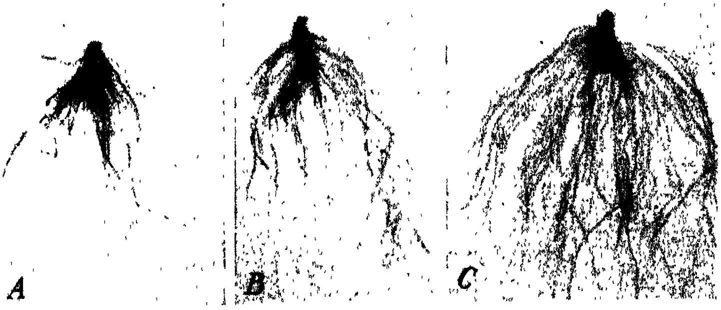

FIGURE 5.—Roots of tobacco plants grown in brown root rot soil: *A*, In moist unaerated soil; *B*, in moist aerated soil; *C*, in soil dried during the period of aeration. Aeration of moist brown root rot soil does not appreciably reduce the amount of disease.

The results of many trials, some of which are shown in table 7, further demonstrate quite conclusively that aeration (fig. 5) or pure oxygen as such has comparatively little if any influence on the persistence of brown root rot in the soil. The effect of drying on brown root rot soil, therefore, cannot be attributed even in small part to direct oxidation of an injurious chemical substance that may be the responsible agent.

TABLE 6.—*Effect of drying as compared with aeration on the causal agent of brown root rot*

[2,000 g of soil in refrigerator pans treated as shown for 30 days]

Treatment of soil	Moisture		Amount [1] of brown root rot after treatments
	Average daily loss	Average daily supply	
	Grams	*Grams*	
Not stirred (pan covered)	1	1	++++
Stirred daily (pan covered)	1	1	++++
Stirred daily (pan open)	40—0	0	0
Do.	40	40	++++

[1] 0=no disease, ++++=heavy

TABLE 7.—*Effect on the causal agent of brown root rot of exposing soil to oxygen, nitrogen, and air for 7 days, with and without drying*

[Atmosphere regulated in closed container and soil stirred frequently]

Atmosphere	Soil condition at end of exposure	Amount [1] of brown root rot after treatment		Atmosphere	Soil condition at end of exposure	Amount [1] of brown root rot after treatment	
		Soil 1	Soil 2			Soil 1	Soil 2
Oxygen	Moist	+++	+++	Nitrogen	Dry	0	+
Nitrogen	do	+++	+++	Air	do	+	+
Air	do	+++	+++	Control (no treatment).		+++	+++
Oxygen	Dry	0	+				

[1] 0=no disease; +=trace; +++=moderate

Some farmers believe it is possible to control the disease in the field by aerating the soil through several deep cultivations prior to planting. Any benefits secured from such cultivation probably result from increased exposure of the soil to drying, a condition which could hardly be expected to be, and in practice evidently is not, effective in seasons of normal rainfall.

It is also conceivable that the exposure of brown root rot soil to sunlight may reduce the disease during drying or aeration experiments, especially where the soil is continually or repeatedly stirred or agitated in the presence of strong sunlight. To test this possibility, brown root rot soils were exposed to drying and to aeration without drying, both in the presence and in the absence of light. No reduction in brown root rot that could be attributed to light was found in these trials (table 8).

TABLE 8.—*Effect of light as compared with drying on the causal agent of brown root rot in soil*

Sunlight	Time of exposure	Soil condition at end of exposure	Amount [1] of brown root rot in—	
			Soil 1	Soil 2
	Hours			
Subdued	24	Moist	+++	+++
Do	24	Dry	0	0
None	24	do.	0	0
Strong	5	Moist	+++	+++
Do	5	Dry	0	0
Control	......	Moist	+++	+++

[1] 0=no disease, +++=moderate.

EFFECT OF CHEMICALS ON THE CAUSAL AGENT

The high susceptibility of the causal agent of brown root rot to certain physical conditions suggests that it also may be intolerant to low concentrations of various chemicals added to the soil. It was especially hoped that some correlation might be found between the reaction of brown root rot to certain chemicals and the germicidal properties of such chemicals. In this respect the results are somewhat conflicting, but wholly satisfactory data are not easily secured in some instances since the concentration of nonvolatile chemicals used must of necessity be below that seriously toxic to the host or test plant used. Silver nitrate, copper sulfate, boric acid, and lithium carbonate, for example, were found to be extremely toxic in low concentrations in the soil. Although brown root rot was not entirely eliminated by the concentrations of these chemicals that could be used, it is likely that somewhat higher concentrations might have been more destructive to brown root rot. The destructive chemicals shown in table 9, though widely different as regards germicidal properties, completely eliminated brown root rot from the soil. Of these, only formalin and acetic acid (*3*) may be regarded as effective soil fungicides of known value, although all the others, with the possible exception of charcoal, possess more or less germicidal properties. The behavior of charcoal is especially interesting and again suggests adsorption of the causal agent as a possible explanation. In the gaseous form, chloroform, toluene, xylol, ammonia, formalin, carbon bisulfide, and ethylene chlorhydrin were all very effective as destructive agents.

Quite as significant is the list of chemicals that failed to affect brown root rot appreciably. Substances that alter the reaction of the soil to a considerable degree (hydrochloric acid, sodium hydroxide, calcium carbonate, and sulfur) may have been expected to give some degree of modification in brown root rot, but this was not apparent although the soil reaction was varied from pH 4.6 to 7.7. Potassium permanganate produced interesting chemical "lesions" on the roots, which, at low concentrations of the chemical, resembled those of brown root rot. The disease lesions were nevertheless also conspicuously present. Other chemicals, e. g., boric acid, may be very toxic to the foliage and stems of tomato without showing marked toxicity to the roots or to brown root rot. The failure of naphthalene vapor and Cynogas to affect the disease offers some presumptive evidence that neither nematodes nor other soil fauna are concerned. The differential behavior of chloroform and ether seemed at first rather significant.

However, when cultures of different organisms were exposed to these chemicals under similar conditions, it was apparent that chloroform inhibited fungi and bacteria much more completely than did ether.

TABLE 9.—*Chemical substances tested on brown root rot at concentrations*[1] *permitting the subsequent growth of tomato plants in the treated soil*

Chemicals destroying causal agent			Chemicals not destroying causal agent		
Chemical	Concentration or physical state	Amount used on 1.2 kg of soil	Chemical	Concentration or physical state	Amount used on 1.2 kg of soil
Alcohol (ethyl)	50 percent	200 cc	Aluminum hydroxide (dry)		24 g
Ammonium hydroxide (c. p.)	Gas	10 cc	Ammonium sulfate	1 percent	200 cc
Acetic acid	1 percent	200 cc	Boric acid	0.5 percent	200 cc
Carbon bisulfide	Fumes	10 cc	Calcium carbonate (dry)		48 g
Charcoal (dry)		24 g	Copper sulfate	0.1 percent	200 cc
Chloroform	Fumes	10 cc	Cynogas (commercial dry)		10 g
Ethylene chlorhydrin	10 percent	200 cc	Ether	Fumes	20 cc
Formaldehyde (40 percent)	Gas	10 cc	Hydrogen peroxide	3 percent	200 cc
Mercuric chloride	0.2 percent	200 cc	Hydrochloric acid	1.5 percent	200 cc
Toluene (c. p.)	Gas	10 cc	Infusorial earth (dry)		24 g
Xylol (c. p.)	do	10 cc	Kaolin (dry)		24 g
			Lithium carbonate	0.2 percent	200 cc
			Naphthalene (dry)	Gas	10 g
			Potassium permanganate	0.5 percent	200 cc
			Sodium hydroxide	0.2 percent	200 cc
			Sodium nitrate	2 percent	200 cc
			Silver nitrate	0.1 percent	200 cc
			Sulfur (dry powder)		4 g
			Talc (dry powder)		24 g
			Zinc sulfate	0.1 percent	200 cc

[1] The treatments with gases and fumes were made in a closed 5-liter container in which the amount of chemical shown was evaporated from a shallow dish.

Although the preliminary results of the experiments with chemicals do not in themselves offer any direct explanation of the nature of brown root rot, they are of some special interest in connection with further means of identification of the causal agent and suggest also possibilities of a practical control of the disease through chemical sterilization of the soils. Further studies should be made of the influence on the normal flora and fauna of the soil of chemicals that inhibit brown root rot. Such investigations should permit the elimination of certain species or groups of micro-organisms as probable agents in the production of the disease.

SUSCEPTIBILITY OF PLANT SPECIES TO THE DISEASE

In the earlier trials reported from this laboratory (*11*), the following plants were named as attacked by brown root rot: Tobacco, tomato, potato, eggplant, pepper, cowpeas, soybeans, garden beans, hairy vetch, and clovers. A considerable variation in relative susceptibility of the plants was noted at the time. Plants found difficult to classify included corn, oats, barley, wheat, rye, and timothy. Species apparently immune included cabbage, onion, beet, pumpkin, cucumber, and lettuce.

In recent experiments, most of these plants and a considerable number of other species have again been grown in brown root rot soil, and their relative susceptibility to the disease has been approximately determined. Such plants are listed in table 10 as showing three degrees of susceptibility; also included in table 10 is a list of plants that are apparently immune according to these tests. The plants tested were selected chiefly with a view to including the more common crop and ornamental plants, a small group of weeds, and another group of the Leguminosae. The legume family was selected for special consideration largely because of the suspected susceptibility of its species to the disease and their common use as soil-improving crops. Despite the number of very susceptible species in this family, preliminary results indicate that there may be some variation in susceptibility between different varieties of the same species, especially in the varieties of *Pisum sativum* L., but further trials are needed to establish this point.

TABLE 10.—*Plant species showing approximate relative susceptibility to brown root rot*

Very susceptible	Susceptible	Resistant	Immune
Tobacco (*Nicotiana tabacum* L.). Tomato (*Lycopersicon esculentum* Mill) Cotton (*Gossypium hirsutum* L). Okra (*Hibiscus esculentus* L). Snapdragon (*Antirrhinum majus* L) Sunflower (*Helianthus annuus* L.). Cornflower (*Centaurea cyanus* L) Bushy arctotis (*Arctotis stoechadifolia* Berg). Leguminosae Cowpea (*Vigna sinensis* (Torner) Savi) Soybean (*Soja max* (L) Piper). Common bean (*Phaseolus vulgaris* L). Common pea (*Pisum sativum* L). Peanut (*Arachis hypogaea* L.). Locust (*Robinia pseudoacacia* L). Sesbania (*Sesbania punicea* (Cav.) Benth.). Fenigreek (*Trigonella foenum-graecum* L.). Tepary bean (*Phaseolus acutifolius latifolius* Freem Civet bean (*Phaseolus lunatus* L) Velvetbean (*Stizolobium deeringianum* Bort.). Hairy vetch (*Vicia villosa* Roth). Vetch (*Vicia grandiflora* Scop , *V atropurpurea* Desf., *V. pannonica* Crantz).	Potato (*Solanum tuberosum* L). Eggplant (*Solanum melongena* L) Pepper (*Capsicum annuum* L). Nightshade (*Solanum miniatum* Bernh) China-aster (*Callistephus chinensis* (L) Nees). Lettuce (*Lactuca sativa* L) Nasturtium (*Tropaeolum majus* L) Common basil (*Ocimum basilicum* L). *Gaillardia* sp California-poppy (*Eschscholtzia californica* Cham.). Marjoram (*Majorana hortensis* Moench) Spurge (*Euphorbia* sp) Leguminosae Lentil (*Lens esculenta* Moench). Alsike clover (*Trifolium hybridum* L.) Alfalfa (*Medicago sativa* L.). Lespedeza (*Lespedeza striata* (Thunb) Hook and Arn). Mung bean (*Phaseolus aureus* Roxb). Pigeonpea (*Cajanus indicus* Spreng.). Partridge-pea (*Chamaecrista fasciculata* (Michx.) Greene) (*Desmanthus illinoensis* (Michx.) MacMil.). Trefoil (*Lotus corniculatus* L.)	Groundcherry (*Physalis pubescens* L) Apple-of-Peru (*Nicandra physaloides* (L.) Pers.). Buckwheat (*Fagopyrum esculentum* Moench). Hemp (*Cannabis sativa* L) Common beet (*Beta vulgaris* L.). Spinach (*Spinacia oleracea* L) Wheat (*Triticum aestivum* L) Timothy (*Phleum pratense* L.) Broomcorn millet (*Panicum miliaceum* L). Rice (*Oryza sativa* L). Sudan grass (*Sorghum vulgare sudanense* (Piper) Hitchc) Quackgrass (*Agropyrum repens* (L) Beauv.) Calendula (*Calendula officinalis* L). Garden balsam (*Impatiens balsamina* L.). Catchfly (*Silene noctiflora* L). Pigweed (*Amaranthus retroflexus* L) Sheep sorrel (*Rumex acetosella* L). Wallflower (*Cheiranthus cheiri* L.). Fennel (*Foeniculum vulgare* Hill). Dill (*Anethum graveolens* L) Nemesia (*Nemesia strumosa* Benth). Gilliflower (*Matthiola* sp). Leguminosae: Sweet pea (*Lathyrus odoratus* L.) White clover (*Trifolium repens* L.). Milkvetch (*Astragalus* sp). (*Lathyrus hirsutus* L). Beggarweed (*Desmodium tortuosum* (Sw) DC. Crownvetch (*Coronilla varia* L) Spring vetch (*Vicia sativa* L.). Vetch (*Vicia picta* Fisch. and Mey).	Jimsonweed (*Datura stramonium* L.). Onion (*Allium cepa* L). Pumpkin (*Curcurbita pepo* L). Cucumber (*Cucumis sativus* L.) Cabbage (*Brassica oleracea capitata* L.). White mustard (*Brassica alba* (L.) Boiss). Barley (*Hordeum vulgare* L). Oats (*Avena sativa* L.). Rye (*Secale cereale* L.) Corn (*Zea mays* L) Bluegrass (*Poa bulbosa* L). Reed canary grass (*Phalaris arundinacea* L). Chicory (*Cichorium intybus* L). Pokeweed (*Phytolacca decandra* L.). Plantain (*Plantago major* L.). Bindweed (*Convolvulus sepium* L.). French weed (*Thlasp arvense* L.). Campion (*Lychnis alba* Mill). Sweet-william (*Dianthus barbatus* L.). Coneflower (*Rudbeckia laciniata* L.). Stock (*Matthiola incana* (L.) R. Br.). Drummond phlox (*Phlox drummondii* Hook). Scabiosa (*Scabiosa atropurpurea* L.) Butterflyflower (*Schizanthus* sp.). Milkweed (*Asclepias syriaca* L) Zinnia (*Zinnia elegans* Jacq.).

The comparative influence of plants immune and susceptible to brown root rot, when used as preceding crops for tobacco, is not yet definitely known. It is only known that the use of certain susceptible plants like tobacco in "diseased" soil neither maintains nor increases the amount of disease in subsequent years, but rather strikingly decreases the disease. This circumstance may, as has been shown, be due in considerable measure to the greater exposure of cultivated soils to the influence of the weather.

The increased host range presented, it is hoped, may aid in the selection of plants for further experimental work. Some of the new hosts should prove especially suitable for new etiological studies.

PATHOLOGICAL HISTOLOGY OF THE DISEASE[4]

Earlier microscopic examination of the exterior of diseased roots and lesions torn apart with needles frequently showed the presence of abundant hyphae of fungi that appeared to be closely associated with the disease. A form of *Rhizoctonia* that was especially common (*11*) was isolated with some difficulty but proved not to be capable of reproducing the disease.

Examination of the diseased tissue by means of freehand and paraffin-sections failed to show either frequent or extensive invasion of the affected cells by fungi of any sort. The yellowing of the cell walls and the early granulation of the cell contents followed by decay of the cells in the late stages of the disease were the only evident histological features present. The failure of other means of study to yield conclusive results on the nature of the disease seemed, however, to warrant a more comprehensive reexamination of the affected tissues by histological methods. This work was undertaken and conducted chiefly with greenhouse-grown material from various soils collected in 1934 and 1935 from Massachusetts, Wisconsin, and Maryland fields, although some further study with improved techniques was made from paraffined material fixed prior to 1926 and as early as 1918. Over 150 separate specimens of paraffined root tissue were cut, stained, and mounted, involving the examination of several thousand sections. In addition, sections were cut from about 60 specimens of roots that had been previously wounded mechanically (by needle punctures, razor cuts, etc.), injured by chemical agents (phenol, ammonium carbonate, etc.), or infected with black root rot (*Thielaviopsis basicola*) for comparison with brown root rot lesions.

Practically all of the histological studies were made on tobacco and tomato roots. Vigorous roots from young plants, with lesions of varying ages, were usually selected. Out of 10 fixatives tried, Karpechenko's gave the best results. Specimens about one-fourth of an inch in length were fixed, washed, and dehydrated in butyl or ethyl alcohol, cleared in cedarwood oil, and embedded in paraffin at 51° to 52° C. Sections were cut 8μ or 10μ thick; staining methods used included 16 single, 5 double, and 2 triple stains. Best results were secured with cyanine and erythrosin prepared according to Chamberlain's general formula for aniline dyes. Mallory's triple stain, methyl green, and Congo red also gave fair results. Typical brown root rot gives early a distinctly yellow or brown coloration to

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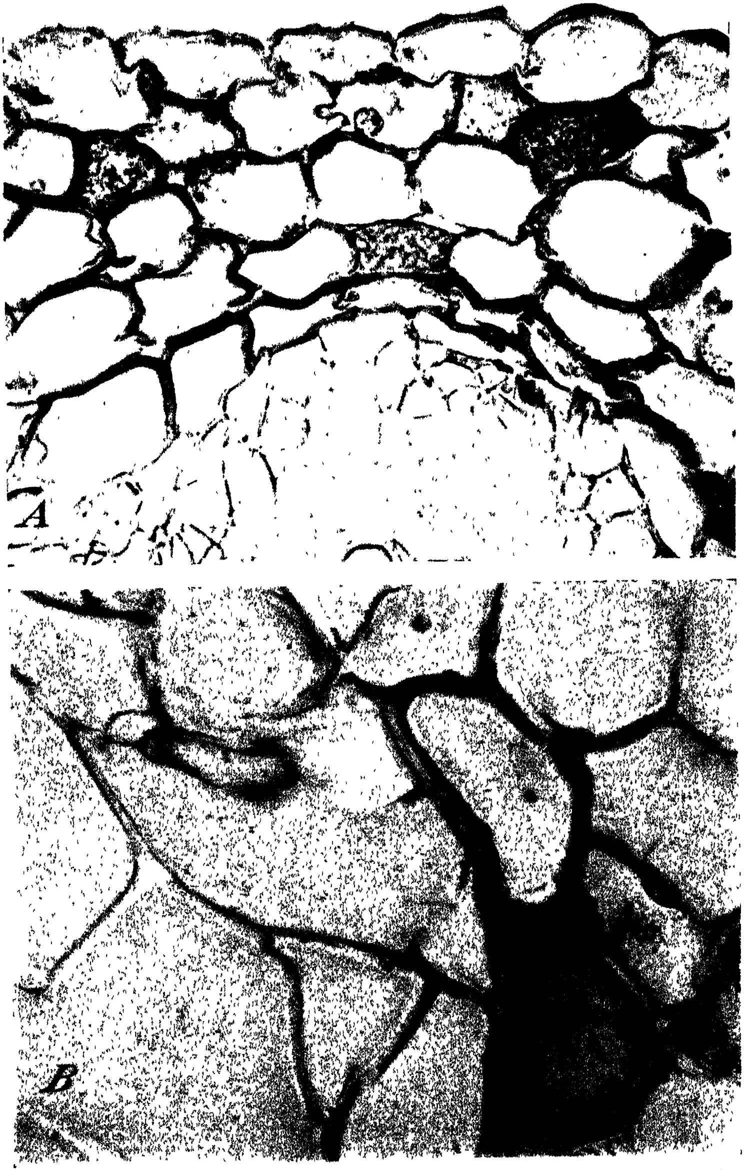
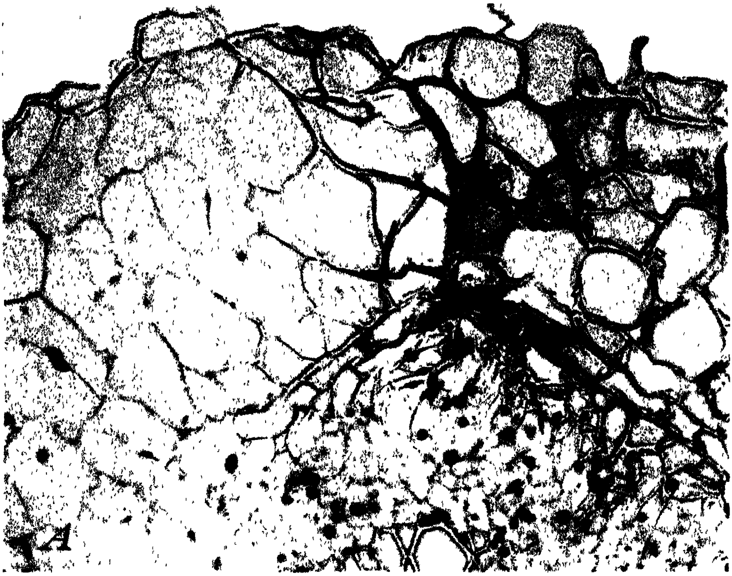
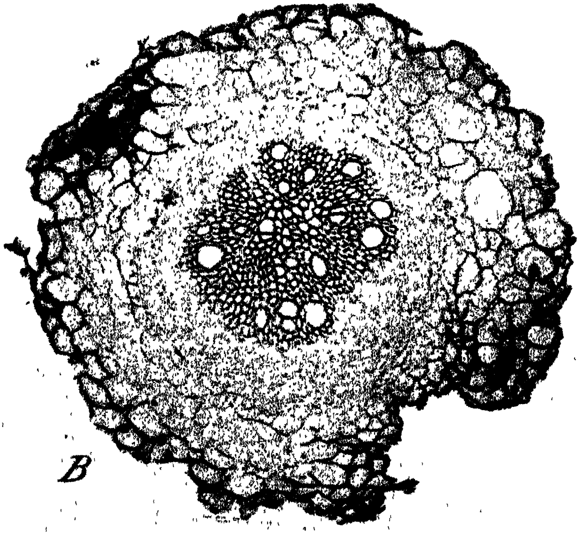
[4] The writer is indebted to Elmer McMurry for able assistance in carrying out this phase of the investigation.



Sections of tobacco root affected with brown root rot. *A*, Early stage of the disease, in which sharply delimited cells filled with finely or coarsely granulated material are conspicuous. Neither in these nor in neighboring cells is there any evidence of a micro-organism regularly associated with the disease. In *B* the contrast between healthy and diseased areas is shown, the cell walls in the latter being yellowed and thickened. *A*, × 500; *B*, × 1,200.





A, Section of tobacco root affected with brown root rot. The disease frequently penetrates the cortex to the endodermal region, where the spread may be more rapid in a lengthwise direction. *B*, Cross section of tobacco root affected with brown root rot, showing injury to epidermal cell layers around entire root, with deep lesions in two areas. *A*, × 500; *B*, × 180.

the cell wall and cell contents, so that it is often desirable to study the sections without staining.

One of the most striking characteristics of brown root rot lesions is the sharp demarcation by the cell walls of the diseased areas from healthy surrounding tissues. In the early stages of the disease or at the margins of the lesions it is not uncommon to find stray single diseased cells in the epidermal layer or deep in the cortical tissue. Examination of the younger lesions under high powers shows the entire cell protoplasm to be disintegrated, only finely or coarsely granular material being present (pl. 1, *A*). Neither in these affected cells nor in cells surrounding them is it possible to find the regular presence of any foreign organism. Occasional fungus strands and nematodes may be seen, but because of their irregular association with the disease, they are evidently secondary invaders. The granular structure of cell material is not suggestive of bacteria nor does it appear that bacteria may be associated with its formation. On the basis of extensive studies of paraffin sections, it appears most unlikely that internal parasitic organisms are concerned with the malady. The possibility of micro-organisms on the root surfaces affecting the root tissues deleteriously through secretions is not obviated by these histological methods.

An outstanding feature of the affected tissue is the thickening of the cell walls (pl. 1, *B*). It is likely that the cell wall is the first to show signs of disease through yellowing, followed by gradual thickening to two or three times its normal width. It has not been possible to observe earlier stages of the disease, either because of the low content of cytoplasm in the root areas used or because the staining has not been suitable for the purpose. The nuclei evidently disintegrate in diseased cells, as shown by their modified reaction to stains, but further intermediate stages of the nuclei or of the cytoplasm that might account for the cell granulations have not been observed.

With this picture of the pathological condition of the cells, it is possible to follow the progress of the disease in the roots to a considerable extent. It is clear from gross examination that a large number of local lesions are normally responsible for extensive root injuries and that brown root rot starts and progresses very slowly. The disease is often plainly initiated at the point of emergence of secondary rootlets, but no evidence has been found that would indicate any limitation to this area since single epidermal cells or small groups of cells elsewhere are not infrequently found to be diseased. From such initial epidermal flecks the lesion may spread in all directions. If the spread is largely superficial, it may involve the periphery to a depth of only one or two cells for some distance. More commonly the lesion spreads laterally through the cortex until the endodermal region is reached (pl. 2, *A*). Here the resistance to the progress of the disease seems to be less than elsewhere, and the stele is soon surrounded; then follows a more rapid longitudinal spread of the disease between the stele and the cortex. This often results in the separation of the cortex from the stele and accounts for the fairly common macroscopic symptom of sloughing off of the living cortex from diseased roots, leaving only the threadlike central cylinder in young roots. In young roots also, before the interfascicular phloem has been developed, the disease occasionally penetrates to the xylem by means of a medullary ray. After secondary phloem encircles the stele, infection rarely penetrates—the phloem acting as a barrier—although occasional pericycle cells and outer secondary phloem are distinctly affected for some distance.

In older roots, the disease is less likely to reach the endodermal region and the lesions may be limited entirely to the cortical layer, causing deep gashes and scars in the roots, which nevertheless may never completely succumb to the disease (pl. 2, *B*). Some evidence of the development of a physical barrier to the progress of the disease has been frequently observed. The development of meristematic tissues in pericycle or cambial layers below the lesion appears to aid in protecting the area of the stele from disease invasion in somewhat the manner described by Conant (*2*) for black root rot. Whatever the normal or stimulated barrier may be, as secondary thickening develops in the roots they become less predisposed to brown root rot and less injury results from developing lesions.

The microscopic examination of brown root rot specimens from the several different soils of the Connecticut Valley, Maryland, and Wisconsin showed very similar pathological histology and suggests the causal agent to be the same in all instances.

It is of some special interest and value in the present connection to compare the pathological histology of brown root rot with lesions produced by more definite causal agents, such as toxic chemicals, mechanical wounds, or parasitic fungi. The treatments were made on free roots grown from the base of potted plants suspended over crocks containing water or the chemical solution used. The roots could therefore be examined at any time for purposes of selecting specimens for fixation. Of many chemicals tested, none was found that would produce external symptoms similar to those of brown root rot, although some at the proper strength produced comparable symptoms. The mechanical injuries on the roots were made by needle punctures, razor cuts, and pinching with tweezers. The parasitic lesions were produced by *Thielaviopsis basicola* on tobacco. Without describing in detail the obvious and characteristic macroscopic differences between lesions produced by the methods described and lesions found in brown root rot, it is worthy of note that close replicas of the histological picture of brown root rot could be found in all types of cell injury, namely, cell-wall discoloration, cell-wall thickening, and granulation of cell contents. In general, the fundamental features of the pathological histology of brown root rot do not appear to be specific for the disease, and while they do not exclude the possibility that a toxin of some sort is involved, the failure to find a microscopically visible organism in or between the diseased cells lends support to the idea that a typical parasite is not involved.

DISCUSSION

The results presented in this paper are chiefly of interest in relation to the nature of the causal agent of brown root rot. Evidence for and against a parasitic cause of the disease was presented earlier (*11*), and both hypotheses have received some support from later workers (*4, 14*). Some phases of the problem remained clearly in need of more detailed study, namely, the behavior and properties of the causal agent and the pathological anatomy or histology of the disease. The studies on properties have shown to a more striking degree than previously the extreme sensitivity of the causal agent to certain environmental conditions. Although the elimination of the causal agent from the soil by sterilizing at 100° C., for example, is suggestive, its destruction at 45° is of much greater significance as bearing on its nature. This and other properties of the causal agent should aid materially in eliminating many organisms suspected of having a causal relation to the disease and should give a more specific idea as to what type of organism or substance may be concerned.

Similarly, the histological picture of the disease, and more particularly the failure to find any evidence of a constantly associated organism in the diseased tissues in a wide variety of material, may be regarded as highly contradictory evidence against a parasitic agent of the usual nature. It does not disprove the possibility that organisms confined largely to the exterior of the roots or harbored only in the soil itself may excrete toxic substances that are necrotically injurious to the roots. That fungi produce toxins has long been known and recently has been studied, perhaps most interestingly by Weindling (*18*). Such an assumption perhaps fits most closely the theory suggested by Thomas (*17*).

Although several investigators have reported that decomposing vegetable matter in or out of the soil produces substances toxic to plant growth and to plant roots, much remains to be explained before this hypothesis can be broadly accepted to explain brown root rot. If this explanation should prove to be true it is likely that a specific organism constantly associated with brown root rot soils dominates the decomposition, forming a product that will produce typical brown root rot symptoms. Such an organism or the toxin it produces will probably show the high sensitivity indicated by the behavior and properties of the causal agent of brown root rot. Some evidence has been secured that certain pure cultures of fungi (*Aspergillus terreus* Thom) will produce materials toxic to roots under certain conditions of nutrition, but thus far no typical brown root rot has been secured. Further work of this general type is one of the few remaining obvious methods of attacking the brown root rot problems.

SUMMARY

The true nature of the causal agent of brown root rot is not known. The present paper is concerned chiefly with the behavior and properties of the causal agent and the pathological histology of the affected tissues.

The causal agent is relatively slow in its action on roots and is not highly concentrated in the soil as judged by dilution experiments. The active principle is very sensitive to certain physical conditions and to some chemical substances. It is intolerant to rapid air-drying of the soil but may withstand prolonged slow drying. It is destroyed by heat at a temperature as low as 45° C. in a period of 24 hours and by moderate freezing temperatures maintained for a relatively long period. Aeration and light do not appear to have any injurious effect. Brown root rot is eliminated from the soil by several chemicals that have germicidal properties, as well as by powdered charcoal, whereas other chemical substances with similar properties have little or no effect.

Tobacco and a wide variety of other plants are susceptible to brown root rot. The legume family is particularly susceptible. Susceptibility to the disease is apparently not correlated with the origin or persistence of the causal agent in the soil. The reduction of the causal agent in the soil is apparently dependent in large part on the exposure of cultivated soils in particular to extremes of heat, cold, and drought.

The histological and cytological examination of diseased tissues failed to show any organism to be constantly associated with the disease. The microscopic features of the disease are chiefly the yellowed and thickened cell walls, discolored and granulated cell contents, and sharply delimited diseased and normal cells. The lesions may often be superficial but generally extend through the cortex of young roots, after which a more rapid and extensive spread may develop around the stele.

The results of the present investigation offer no direct proof of the nature of the causal agent of brown root rot but should aid materially in the conduct of further studies on the etiology of the disease.

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RELATION OF THE STRUCTURE OF THE CHALAZAL PORTION OF THE COTTON SEED COAT TO RUPTURE DURING GINNING[1]

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INTRODUCTION

During the ginning of cotton it frequently happens that when a pull is exerted upon the tuft of fibers located at the chalazal (rounded) end of the seed, the seed coat ruptures and a fragment of the seed coat, with its attached fibers, is carried with the lint (pl. 1, *B*).

Foreign matter in lint constitutes waste most of which must be removed during the manufacturing processes. The presence of a considerable quantity of foreign matter lowers the spinning quality or value of the lint, since it adds to the difficulty and cost of manufacturing the raw cotton into yarn and may seriously affect the quality of the finished yarn and cloth.

The chalazal fragments (pl. 1, *B*), together with other seed-coat fragments of various sizes that have arisen from the cutting of seeds by the teeth of the gin saw and from motes (partly developed seeds) that have been crushed during ginning, constitute a common kind of foreign matter. These particles are particularly obnoxious, for their complete removal during manufacture is difficult. The smaller particles cling tenaciously to the lint. The larger particles, which because of their size might otherwise be more easily removed, become partly broken up during carding and combing, thus adding to the number of small particles already present. Small particles that escape the carding and combing action may become incorporated in the yarn, producing imperfections in the form of knotty bulges, dark specks, or neplike structures (pl. 1, *A*). These particles are still apparent in the woven cloth, and in dyeing cause further difficulty (*9*)[3] as their fibers may present the same problem as do neps, sometimes dyeing darker and sometimes lighter than the surrounding background. Thus, any factor that influences the extent of chipping from the chalazal end of the seed affects the spinning quality of the cotton.

It has been shown that the moisture content of the seed cotton at the time of ginning has some influence upon the number of chalazal fragments occurring in the lint and that varieties differ in the extent to which their seeds are likely to chip.[4]

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[1] Received for publication September 19, 1938. This study is part of a program of work on cotton utility and standards research under the leadership of Robert W. Webb, principal cotton technologist, Bureau of Agricultural Economics.

[2] Appreciation is expressed to G. M. Armstrong, of the South Carolina Agricultural Experiment Station, through whose co-operation the plant material upon which this paper is based was grown and periodically tagged.

[3] Italic numbers in parentheses refer to Literature Cited, p 872

[4] Smith, W. S., and Pearson, Norma L. FRAGMENTS FROM THE CHALAZAL END OF THE COTTON SEED, THEIR FORMATION, AND SOME OF THE FACTORS AFFECTING THE EXTENT TO WHICH THEY ARE PRESENT IN GINNED LINT. Unpublished manuscript.

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The present paper describes the structure of the chalazal end of the cotton seed and suggests one explanation as to why seeds chip at their chalazal ends.

MATERIALS AND METHODS

The materials used in this study represented stages in the development of ovules and seeds of three cottons, Super Seven and Acala (*Gossypium hirsutum* L.) and Pima (*G. barbadense* L.). Although these cottons were not originally grown and preserved for this particular problem, it was thought that they represented a sufficient range in variety and species to form a basis for conclusions regarding the characteristics of the chalazal tissues of the cotton seed.

The plants were grown at Clemson College, South Carolina. Unopened flowers were tagged the day before flowering and were labeled as of the day of flowering. Tagging was begun the last of July and continued at 2- or 3-day intervals until the middle of September. When the first tagged bolls were mature, material from different tagging dates, representing different stages in the development of ovules and seeds, was gathered, and portions were preserved in one or more of several different fixatives—Flemming medium, medium chromoacetic, formal-acetic-alcohol, and formalin alcohol. All of the young material (buds to 10-day-old seeds) and portions of each of the older stages were embedded in paraffin in the usual manner. The remaining material was left in 70-percent alcohol. Young stages and certain of the older ones were stained with Flemming's triple stain. Other sections of the older material were mounted unstained or were stained with eosin, gentian violet, safranine, and orange G, alone or in various combinations. Mature seeds also were gathered and dried.

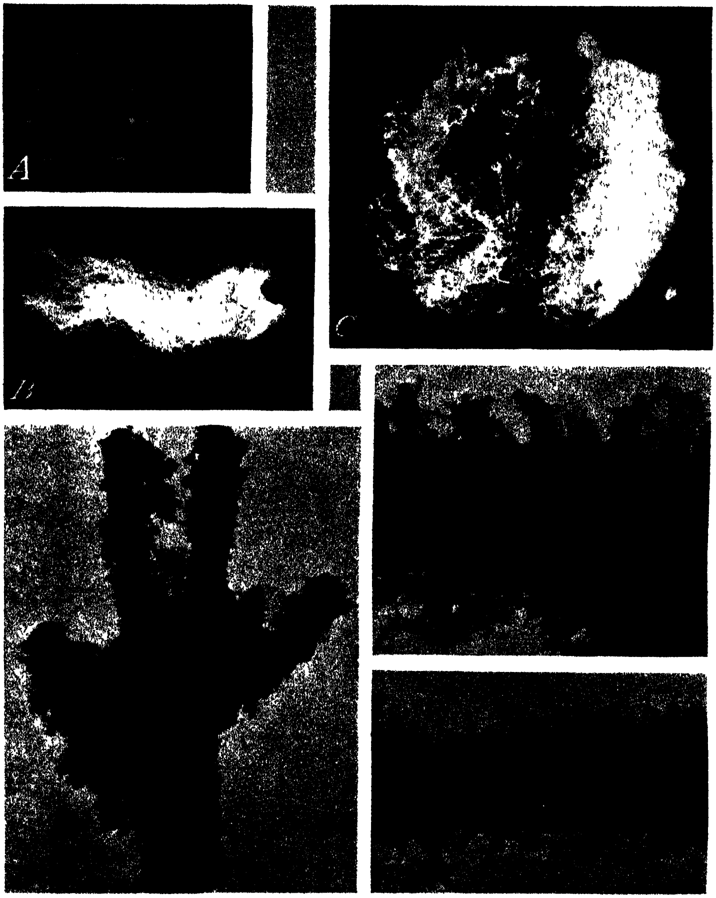
Considerable difficulty was encountered in obtaining unbroken sections of the chalazal end of seeds older than about 30 days. The tissue at the chalazal end of the seed is very brittle in its natural state and when hardened, as is necessary in the process of fixing and embedding, this brittleness is undoubtedly accentuated.

OBSERVATIONS AND CONCLUSIONS

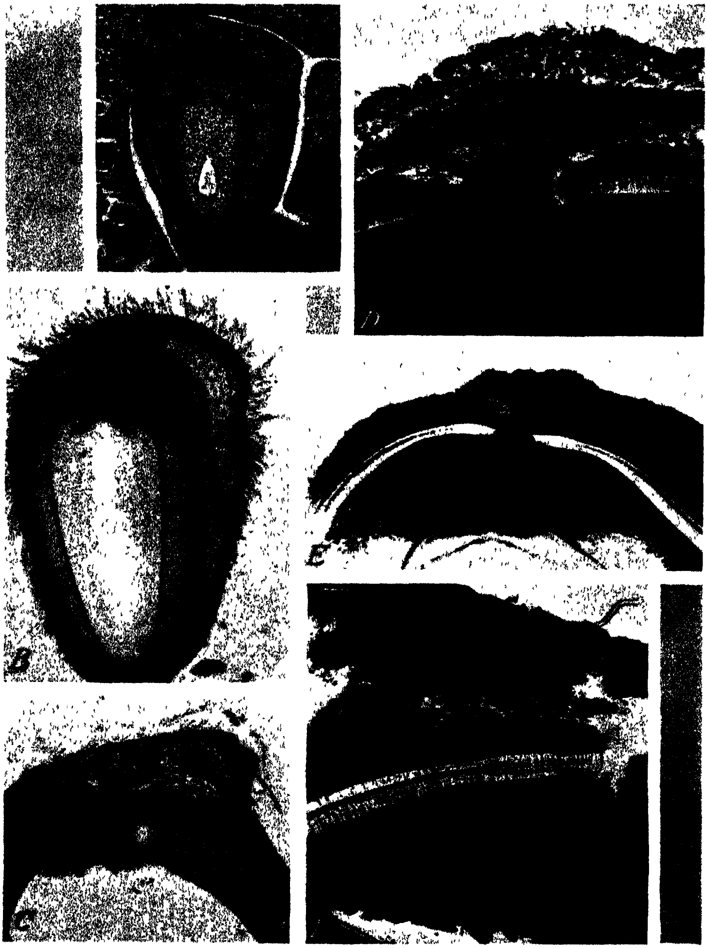
The general structure and development of the cotton seed and cotton seed coat, as revealed by these studies, are essentially the same for Acala, Super Seven, and Pima. There is some evidence of slight differences in rate of development of certain tissues, yet seeds of equal ages from one cotton also exhibit similar slight differences.

As the cotton ovule, its development into a mature seed, and the structure of the mature seed coat have been described and discussed by other investigators (*1, 2, 5, 6, 7, 10, 11, 13*), only a brief description, sufficient for orienting the following discussion, will be presented.

The ovule (figs. 1, *A;* pl. 2, *A* and *B*) is anatropous. Thus, the chalazal end of the ovule is the end farthest away from the point of attachment of the ovule to the placenta, and the micropylar end is nearest. Two integuments arise from the chalaza and surround the nucellus. At the micropylar end, the outer integument overlaps the inner one to such an extent that the micropyle is not a straight passageway between the lips of the two integuments (fig. 1, *A;* pl. 2, *A*). A ridge runs along one side of the ovule. This ridge is the raphe and is formed by the fusion of the funiculus with the outer integument.



A, Irregularities in yarn due to seed-coat fragments. × 2. *B*, Chalazal fragment in ginned lint. × 2½. *C*, Chalazal region of a seed, showing the area from which a portion of the seed coat has been broken away. Note the vascular strands. × 9½. *D*, Vascular strands and surrounding spongy tissue that have been dissected away from a seed similar to *C*. × 36. *E*, Spongy tissue surrounding dissected chalazal vascular strands of 37-day-old Acala seed. × 127. *F*, Same as *E* only from a mature seed of Super Seven. × 127.



A, Longitudinal section through young ovule sectioned at right angles to raphe, Super Seven. × 42. *B*, Longitudinal section through a 2-day-old seed, Super Seven, × 33. *C*, Chalazal portion of a longitudinal section through a 12-day-old seed (fig. 2, *A*), Super Seven. × 21. *D*, Chalazal region of a section through a 22-day-old seed, Acala (fig. 2, *B*). × 42. *E*, Chalazal region of 26-day-old seed, Acala (fig. 2, *C*). × 21. *F*, Portion of chalazal region of 51-day-old seed, Pima. × 60.

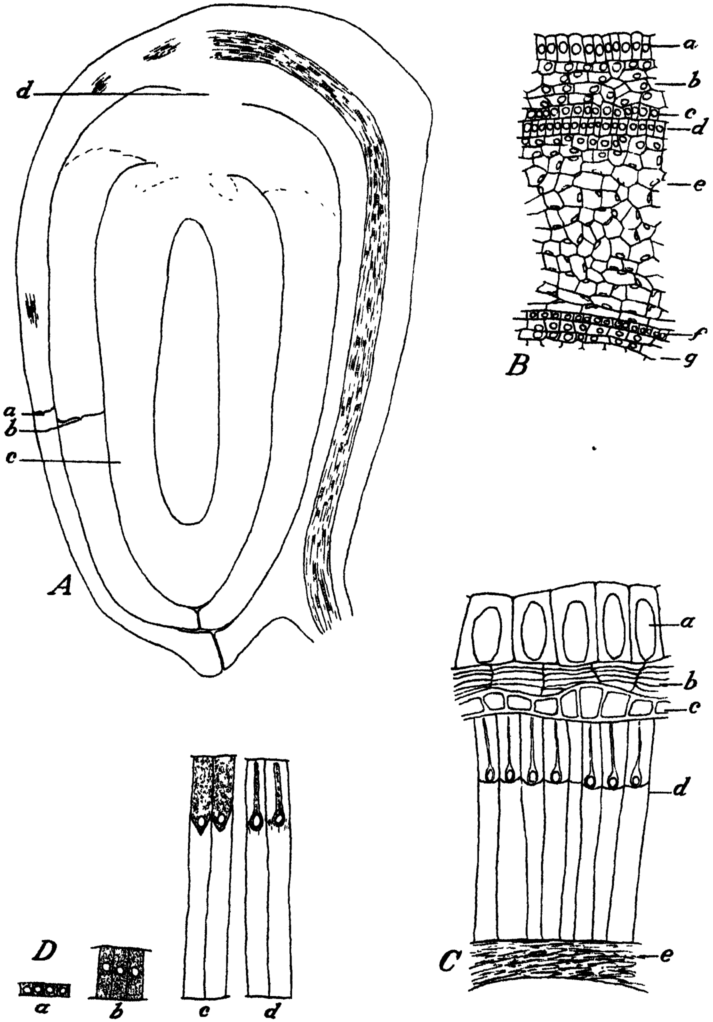


FIGURE 1.—*A*, Diagrammatic longitudinal section through a 1- to 2-day-old seed: *a*, Outer integument; *b*, inner integument; *c*, nucellus; *d*, chalaza. *B*, Section through the integument of ovule on the day of flowering: *a*, *b*, and *c*, Outer integument; *a*, outer epidermis; *b*, parenchymatous tissue; *c*, inner epidermis; *d*, *e*, and *f*, inner integument; *d*, outer epidermis; *e*, parenchymatous tissue; *f*, inner epidermis; *g*, nucellar tissue, × 207. *C*, Section through mature seed coat (semidiagrammatic); *a*, epidermis; *b*, outer pigment layer; *c*, colorless layer; *d*, palisade layer; *e*, inner pigment layer, × 104. *D*, Four stages in development of palisade layer; *a*, 1-day-old seed; *b*, 14-day-old seed; *c*, 28-day-old seed; *d*, 48-day-old seed.

The seed coat of the mature cotton seed develops from the two integuments. Before differentiation takes place, each integument consists of an outer and inner epidermis, and between them there is parenchymatous tissue (fig. 1, *B;* pl. 2, *A;* pl. 3, *C*). The thickness of the parenchymatous tissue varies somewhat at different positions on the seed (pl. 2, *B*). In the outer integument it is traversed by fibrovascular strands.

On the day of flowering or during the next few days (*3, 8*) the outer wall of certain cells of the outer epidermis of the outer integument begins to swell. These swellings elongate and eventually develop into the fibers that cover the mature seed. The epidermal cells that do not develop fibers enlarge considerably and in the mature seed coat are rather irregularly shaped (in surface view) with very thick walls and dark-colored contents.

Beneath the epidermis of the mature seed coat are, successively, the outer pigment layer, the colorless or crystal layer, the palisade layer, and the inner pigment layer (fig. 1, *C*; pl. 3, *D*).

The outer pigment layer, socalled because of the dark contents of its cells, consists of the compressed, somewhat disorganized parenchymatous tissue of the outer integument (fig. 1, *C, b*).

The colorless layer is derived from the inner epidermis of the outer integument (fig. 1, *C, c;* pl. 3, *D*). It is usually one, although occasionally two, cells in thickness. The cells have thick walls, and frequently crystals of calcium oxalate occur in the cell cavities (*6*).

The palisade layer gives strength and hardness to the seed coat. It develops from the outer epidermis of the inner integument (fig. 1, *B, d;* pl. 3, *C*). The cells of this epidermal layer begin to elongate in a radial direction at about the twelfth day after flowering (*1, 13*) (fig. 1, *D*), and eventually a layer is formed that in mature seeds varies in thickness from 146μ to 243μ, depending upon the species or variety (*4, 7*) and perhaps upon environmental conditions as well. About the twenty-first day after flowering, the walls of the elongating cells begin to thicken at their inner ends (*1, 13*). This thickening continues outwardly until a solid deposition is formed in approximately the inner two-thirds of each cell (fig. 1, *D, c;* pl. 3, *D*). In the external third, secondary thickening is laid down in the form of rounded ridges (*11*), leaving a small lumen that is somewhat larger at its inner end where the nucleus lies (fig. 1, *D, d*).[5] In mature seeds, the palisade layer is strongly lignified, the exact distribution of the lignin differing somewhat with different species (*7, 11*).

The inner pigment layer is derived from the parenchymatous tissue of the inner integument. It resembles the outer pigment layer, in that its cells have dark contents and are somewhat crushed and disorganized. For the most part it is thin, but at the chalazal end it is much thickened and forms a little cushion (pl. 2, *E*) (*2, 6, 11*).

The inner epidermis of the inner integument forms a layer known as the "fringe" layer. This layer does not belong to the seed coat proper, but together with the perisperm and the remains of the endosperm, forms a sheath that surrounds the embryo. The cells of the mature fringe layer are somewhat elongated, and there are projections on their walls that extend into the lumen (*6, 11*).

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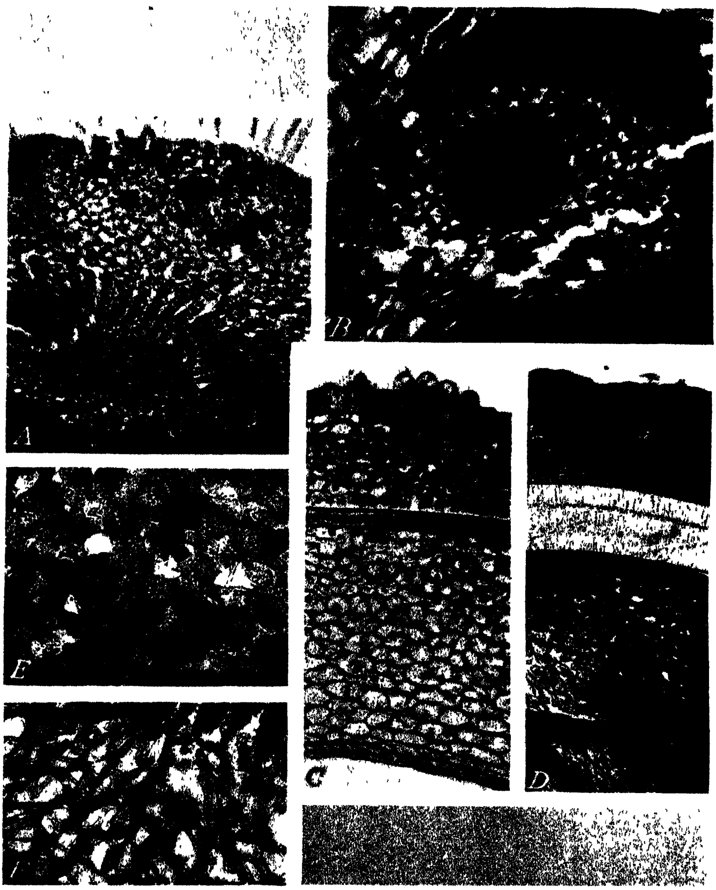
[5] It has been reported that the ratio between the lengths of the inner and outer portion of the palisade cells of mature seeds may vary with species and varieties (*4*).



A, Chalazal region of a section through a 7-day-old seed, showing the more or less distinct strands of linearly arranged cells associated with the chalazal plug (the section is not a true longitudinal one), Super Seven. × 125. *B*, Vascular strand in the chalazal region, surrounded by spongy tissue (pl. 2, *C*), Super Seven. × 94. *C*, Section through testa of a 2-day-old seed (fig. 1, *B*), Pima. × 219 *D*, Section through seed coat of a 41-day-old seed (fig. 1, *C*), Pima. × 63. *E*, Chalazal spongy tissue of an 8-day-old seed, Pima. × 219. *F*, Chalazal spongy tissue from 22-day-old seed, Pima. × 94.

Earlier investigators considered the fringe tissue to have arisen from the epidermis of the nucellus (*2, 6, 13*), but recent work of Reeves (*10*) has shown that this layer is formed from the inner epidermis of the inner integument and cannot be considered as belonging to the perisperm. The author's investigations confirm the findings of Reeves.

The description given above applies to the greater portion of the mature seed coat. The chalazal portion, however, is somewhat different in structure. In a view of the chalazal region of a longitudinal section through a mature cotton seed, the characteristic that stands out most strikingly is that the palisade layer is not continuous. It ends abruptly, leaving a very small round opening (fig. 2, *B* and *C*; pl. 2, *E*). The structure of the portion of the palisade layer surrounding this opening is peculiar. The individual cells in certain definite portions differ in length from the cells in certain other portions, resulting in variations in the thickness of the palisade layer. As the layer curves up over the broadened part of the seed, it gradually becomes thinner (the individual cells being progressively shorter) and then nearer the opening thickens somewhat (the individual cells being progressively longer) (fig. 2, *C* and *B*; pl. 2, *E* and *F*). This thickened part extends for a short distance before the layer becomes slightly thinner and then ends abruptly (pl. 2, *F*).

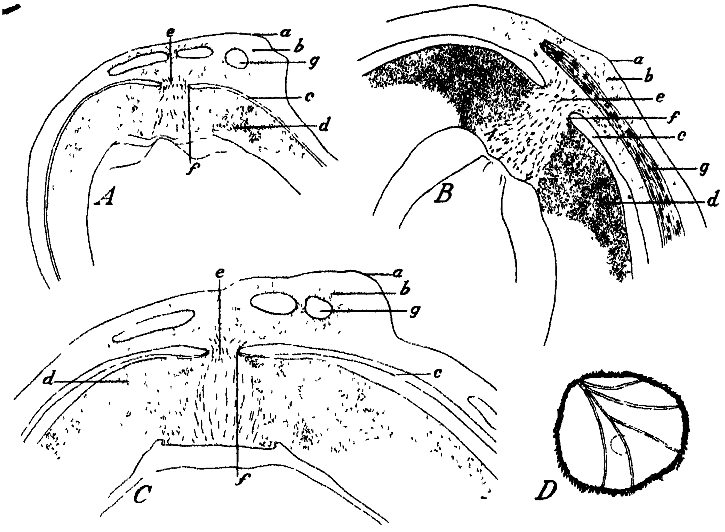


FIGURE 2.—*A*, *B*, and *C*, Chalazal portions of longitudinal sections through seeds (semidiagrammatic), × 21: *A*, 12-day-old seed *B*, 22-day-old seed. *C*, 32-day-old seed. *D*, Vascular strands in the chalazal portion of the seed, seen after dissecting away the overlying tissues. *a*, Epidermis; *b*, outer chalazal spongy tissue (dotted); *c*, palisade layer; *d*, inner spongy tissue (dotted) of chalazal cushion; *e*, chalazal plug; *f*, strands of linearly arranged cells; *g*, vascular tissue.

In addition to the variations in length of the cells composing the chalazal portion of the palisade layer, there is also a variation in the amount of solid deposition in the inner portion of each cell. Passing toward the opening from the region where the palisade layer first grows thinner, the solid portion in each cell is seen to grow progressively shorter until, in the thickened portion near the opening, it occupies only about the inner one-fourth or one-fifth of each cell (fig. 2, *C*; pl. 2, *F*).

The opening in the palisade layer is nearly filled with a core of compact undifferentiated cells (pl. 2, *C*, *D*). This core has been called, for the sake of convenience, the chalazal plug.

The explanation for the opening in the palisade layer is to be found in the structure of the ovule. The palisade layer is derived from the outer epidermis of the inner integument. This epidermis ends at the point where the integument arises from the chalaza. Consequently the palisade layer ends at the chalaza. The chalazal plug thus represents tissue of the chalaza proper.

Examination of the seed-coat tissues reveals that the fringe tissue and the colorless tissue, both of which are derived from epidermal tissues, end at the chalaza.

Between the abrupt ending of the palisade layer and the solid tissue of the plug is a ring of loosely arranged cells, one cell in thickness (pl. 2, *D*). This ring flares out to the inside and to the outside of the opening left by the ending of the palisade layer, forming a sort of sheath made up of more or less separate strands of linearly arranged cells. This linear arrangement of the cells can be most easily demonstrated in young material, for in mature seeds the cells crumble in sectioning. If young seeds are cut longitudinally and to one side of the median of the plug, the separate rows of cells usually can be seen (pl. 3, *A*).

The sheath composed of linearly arranged cells, together with the tissue of the plug, merge externally and internally with tissues which, though corresponding in position to the outer and inner pigment layers, differ in structure from these layers at other parts of the seed coat.

It has been said previously that the inner pigment layer is very much thickened at the region of the chalaza and forms a little cushion (*2*, *6*, *11*). The tissue of this cushion is spongy (pl. 3, *D*) except in the part extending directly between the chalazal plug and the nucellus. The individual cells of the cushion have been described as star-shaped (*2*, *6*). Even in very young seeds, the chalazal tissue that will form this cushion is characterized by the dark contents of its cells (pl. 2, *B*) and by the presence of small intercellular spaces.

The chalazal cushion connects by means of the chalazal plug and its surrounding rows of cells with the tissue external to the palisade layer. This external tissue is even more spongy than the tissue of the cushion (pl. 2, *C*) and will hereafter be designated as the "spongy" tissue.

Very young seeds give evidence of the eventual spongy character of this tissue. Seeds 1, 2, or 3 days old show the tissue in this region to be less compact than the adjacent parenchymatous tissue of the outer integument. The cells are rounded, and small intercellular spaces occur. As the seeds develop, these intercellular spaces increase in size in such a way as to make a tissue the cells of which, though frequently rather elongated, are somewhat star-shaped, each cell possessing several arms that connect with similar arms from adjacent cells (pls. 3, *E* and *F*; 1, *E*).

As the development of the seed progresses, the starlike form of the cells of the spongy tissue becomes more and more pronounced. The cell walls remain thin; and when the mature seed dries, the cells shrivel to a certain extent but still retain to a greater or less degree their star-shaped appearance (pl. 1, *F*). A similar type of development is also taking place in the tissue that will form the chalazal cushion in the mature seed; but the intercellular spaces here do not become so large, nor the projecting arms so delicate, as in the outer tissue.

In the immediate vicinity of the chalazal plug, most of the tissue (exclusive of the vascular strands) between the palisade layer and the epidermis is composed of spongy cells (pl. 2, *C*). The region occupied by these cells extends entirely to or within two or three cells of the epidermis and within a few cells of the palisade layer; it extends outwardly beyond the chalazal plug for varying distances, becomes thinner, and gradually merges with the more solid tissue of the outer pigment layer (pl. 2, *C* and *D*).

The chalazal epidermis is not essentially different from the epidermis of the rest of the seed coat except that stomata and fibers are more numerous (*12*) than elsewhere on the seed.

The single vascular strand that extends through the raphe branches at a point a little to one side of the chalazal plug into several strands that fan out through the chalazal spongy tissue and extend onward through the outer pigment layer (outer integument). Thus in the vicinity of the chalazal plug and for various distances around it, the vascular strands are surrounded by cells of the spongy tissue (pls. 1, *D;* 2, *C;* 3, *B*).

By comparing the position of the spongy tissue in unbroken seeds with the position of the broken area in seeds that have undergone chalazal chipping during either hand ginning or machine ginning, it is obvious that the fragments pulled away include a portion of the spongy tissue. Moreover, the chalazal fragments found in ginned lint show the ragged edges of the broken cells of the spongy tissue, indicating that it is in the spongy tissue that the main rupture occurs.

The broken areas vary in size in different seeds, but careful examination shows that they usually are very close to or include the region over the chalazal plug. Occasionally chipping occurs at the chalazal end of the raphe, and the broken area may include the point at which the raphe strand branches.

If chipping occurs when the seed coat is moist, the vascular strands frequently are left more or less intact on the seed (pl. 1, *C*), indicating that the ruptures occurred in the spongy tissue between the vascular strand and the epidermis. But with seeds that were chipped when the seed coat was fairly dry, the vascular system occasionally may be left fairly intact. Usually, however, it is either entirely torn away or only small fragments are left, the rupture in such cases occurring in the inner portion of the spongy tissue; that is, between the vascular strands and the palisade layer.

Undoubtedly, the spongy tissue constitutes a weak place in the chalazal portion of the cotton seed coat, and its existence furnishes one explanation why cotton seeds rupture at their chalazal ends during ginning.

It must not be overlooked, however, that there may be factors other than the existence of this spongy tissue that aid in bringing about a rupture of the seed coat. The epidermis itself is undoubtedly weakened by the greater number of both fibers and stomata at the chalazal end of the seed (*12*). Thus, an original rupture in the epidermis resulting when force is applied to the attached fibers, might extend beyond the epidermis to the easily broken layer below it, pulling away a fairly large layer of the chalazal epidermis with the attached fibers and parts of the spongy tissue and vascular system.

SUMMARY

During ginning, fragments of the cotton seed coat with the attached fibers may be broken from the chalazal end of the seed, thereby adding undesirable foreign matter to the ginned lint.

Material representing different stages in the development of the seeds of three cottons—Super Seven and Acala (*Gossypium hirsutum* L.) and Pima (*G. barbadense* L.)—showed that the chalazal part of the seed coat has a very characteristic structure.

The palisade layer—since it is derived from the outer epidermis of the inner integument—ends at the point of attachment of the inner integument to the chalaza. Thus a small opening is left in the palisade layer, which is nearly filled with a fairly compact mass of undifferentiated cells, the chalazal plug.

The inner and outer pigment layers are thicker in the chalazal region than elsewhere on the seed and are spongy in nature. The outer pigment layer is much more strikingly spongy than the inner pigment layer, the intercellular spaces being larger and the connecting arms more delicate. The spongy portion of the outer pigment layer extends over the chalazal end of the seed for various distances, eventually merging with the more solid tissue comprising the rest of the layer. This outer spongy tissue is traversed by branches from the single vascular strand of the raphe.

In chalazal chipping, the main rupture occurs in the spongy tissue—either above or below the vascular strands. The broken areas vary in size in different seeds, but usually include a portion of the spongy tissue very close to or over the chalazal plug.

This outer spongy tissue constitutes a weak place in the seed coat and its existence furnishes one explanation for the chipping of cotton seeds at the chalazal end during ginning.

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GROWTH OF THE HEN'S OVUM[1]

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PROBLEM

Considerable study has been made of the hen's ovum during development; however, the record of its growth is far from complete. Insofar as the writers have been able to determine the earlier observations were made from autopsies or after the feeding of the dye, Sudan III. Autopsies obviously provide a rather inaccurate method of measuring ovum growth, and the fact that the interval between feeding and actual deposition of the dye in the ova was not definitely known limited the value of such studies.

Stieve (*13*)[2] constructed theoretical growth curves for the hen's ova by removing and weighing all of the more mature ova of individual hens. Riddle (*8*), Romanoff (*11*), and Marza and Marza (*4*) have given some attention to the hen's developing ovum, and Bissonnette and Zujko (*1*) studied the development of the ovum of the starling. However, in most of the earlier studies emphasis was placed on stages of growth prior to those considered here and the technique utilized was not such as to give critical information of rate of growth. The period here considered is primarily the late stage of greatly accelerated growth which Riddle (*9*) described as starting when the ovum has a diameter of about 6 mm and having a growth rate as much as 25 times that of the previous period.

In the domestic hen ovulation occurs more or less regularly and continuously over long periods. Facts related to the growth of the ovum, therefore, have a bearing on the rate of ovulation and yolk size, which in turn influence the size of the fully formed egg. The physiological basis of differences in rate of laying and egg size was sought in this study.

STOCK AND METHODS

Most of the birds used in this study were White Leghorns, although a few Rhode Island Reds and first-generation hybrids were included. During the course of the experiment the hens were usually kept in individual hen batteries, where they were readily available for injection. The birds used were in their first or second year of production.

It was planned to study the growth of the hen's ovum by use of a fat-soluble dye such as that used by Riddle (*7*) and described more fully by Gage and Gage (*2*) and Rogers (*10*). The junior author suggested the possibility of injecting the dye directly into the blood stream of the bird, thus making possible more accurate time studies. In the studies of earlier workers the dye was fed.

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[2] Italic numbers in parentheses refer to Literature Cited, p. 893.

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A number of fat-soluble dyes were tested, but all were found less satisfactory than the Sudan III utilized by earlier workers. Very few of the fat-soluble dyes were deposited in the ovum. If deposited, they were so lacking in intensity as to give a poorly defined band of color. Sudan IV was nearly as satisfactory as Sudan III.

The dye was prepared for injection by forming a colloidal dispersion in water, by a method similar to that commonly used for substances soluble in alcohol but insoluble in water. A saturated solution of Sudan III in chloroform was first prepared, since the dye is much more soluble in chloroform than in alcohol. The chloroform solution was diluted with alcohol only as it was used, because the alcohol solution formed seemed to be supersaturated, and much of the dye crystallized out on standing. Approximately 4 cc of this solution was diluted with about 10 times its volume of alcohol, and this mixture was poured very slowly into 200 or 300 cc of boiling distilled water. After the chloroform and alcohol had boiled away, more dye was added until a satisfactory concentration was reached. After the last of the alcohol was boiled away, the red colloid was passed through filter paper while still hot. The concentration of dye was determined by diluting a sample with exactly 9 volumes of alcohol and comparing it on the colorimeter with a standard solution of Sudan III in 90-percent alcohol. It was intended that the dye should always be more concentrated than necessary at this point, so that after the concentration was determined, it might be diluted to the standard concentration, which in this case was 0.4 mg per cubic centimeter.

The usual injection was made with 2 mg of the dye in 5 cc of water. It was at first believed that adding daily this quantity of water to the blood stream would be harmful to the bird and thus interfere with egg production. However, the use of even greater quantities seemed in no way to harm the bird. Physiological salt solution was not used since it would have precipitated the dye.

In preliminary experiments to determine the most satisfactory dosage of dye, the quantity of Sudan III injected at a time was varied from 2 to 27 mg. The dosages 2, 4, 8, 14, and 27 mg gave bands of dyed yolk (in the same hen's egg) having mean widths of 0.39, 0.51, 0.63, 0.75, and 1.02 mm, respectively. Although the widths of the bands of dyed yolk did not vary proportionately, they did vary in the direction of the dosages.

Access to the blood stream was gained through the vena humeri profunda and its branches, a vein on the underside of the wing. This is the only vein which is large enough and exposed for sufficient length to meet the needs for repeated injections. By starting at the terminal branches of the vein and alternating between the two wings, as many as 16 daily injections could be made with ease. The chief difficulty in repeating the injections is the discoloration of the tissues due to hemorrhage, which makes the vein difficult to locate. A small nick was cut in the skin immediately over the vein, and a 1-inch (26-gage) needle was inserted in the vein. It was found that bending the needle at about a 30° angle greatly facilitated penetration of the vein. Two persons are required for the operation since the slightest move by the bird will tear out the needle and hemorrhage will obscure the vein for considerable distance. Straps attached to a table are fastened firmly about the bird's thorax and legs thus reducing the opportunity for struggling.

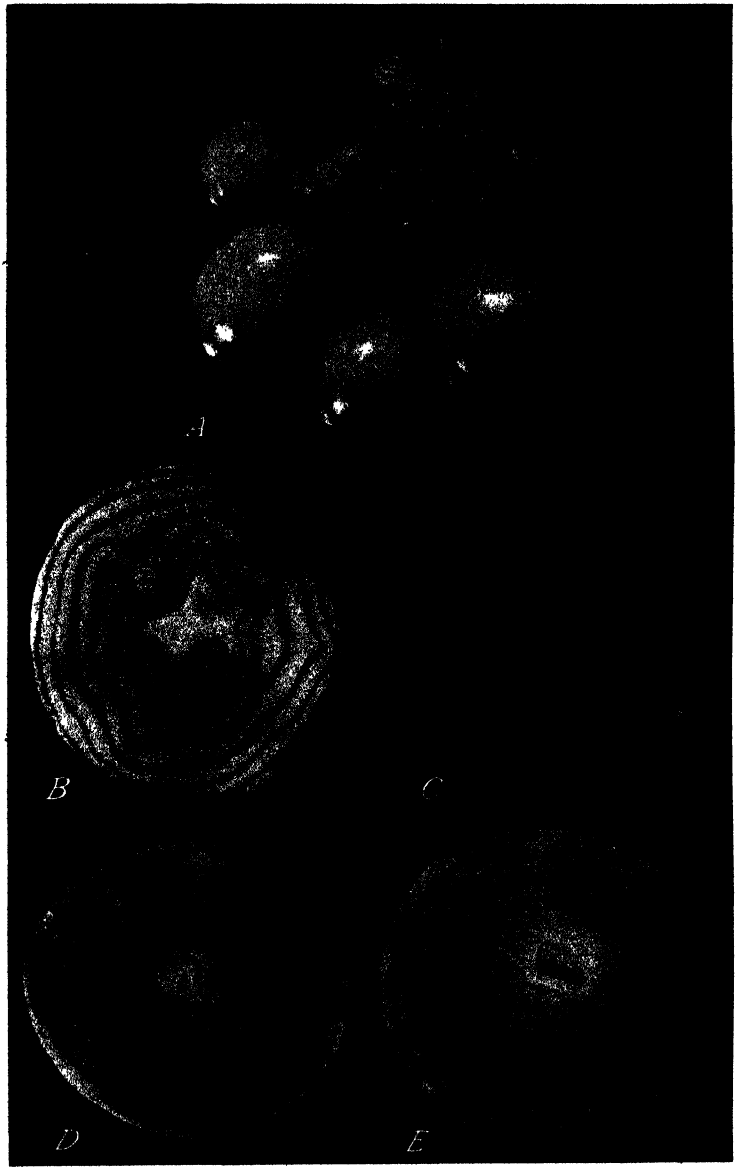

FIGURE 1.—Yolk secretion: *A*, Functional ovary showing gradations in size of immature ova. Above are two recently ruptured follicles. *B*, Transverse section of a boiled yolk showing distortion of inner dye bands. *C*, Note the sharp depression of the dye bands at the neck of the latebra. *D*, *E*, Two yolks showing dye bands resulting from injections at 24-hour intervals. Note the neck of the latebra showing as a break in the dye bands.

Injections were made at uniform intervals, usually 24 hours, the time of day depending on the nature of the experiment. The distance between color bands (fig. 1, *D*, *E*) was used as a basis of measuring growth rate. This technique made it possible not only to calculate the rate of growth at any point in the yolk but also to estimate accurately the size of the yolk at various periods previous to ovulation. In measuring growth the eggs from injected birds were boiled for 20 minutes; the yolks were weighed and halved at the germinal disk with a razor blade. A thin section was taken from the cut surface for measuring the distance between dye bands. A microscope with an eyepiece micrometer and mechanical stage was used for the measuring. The section of the yolk was placed on a slide and adjusted so that the zero reading was on the periphery. Measurements were then taken on the positions of all color bands in the yolk, recording the scale reading for the inner surface of each band. Readings were taken at two positions on the same section of the yolk, one opposite and one at a right angle from the germinal disk, so as to correct irregularities in the bands. The distances of the bands were expressed as the mean of the two measurements. The use of a drop of glycerin on the cut surface sometimes aided in differentiating the dye bands resulting from injections at 12-hour intervals. Readings must be taken immediately upon application of the glycerin since it rapidly disintegrates the yolk. The ovum growth for the interval of time under investigation was represented by the radius increment as measured from the inside surface of one band to the corresponding position on the succeeding band. The inside surface of the band was much more sharply defined than the outside and was a relatively accurate measure of yolk size at the time of injection.

In each injection series, measurements were started as soon as dye appeared in the laid eggs, usually the second day following the injection, and continued as long as the dye rings persisted. Thus eggs at the beginning of a series would have bands only on the outside of the yolk, and those at the close of the series would have them near the center only. Eggs at the middle of the series would have bands throughout. In most experiments, 11 daily injections were made.

In order to determine how soon the dye was deposited in the yolk, hens were killed at varying short intervals after injection, and the entire ovary was removed and boiled. In one hen, dye was unmistakably detected in a half-grown ovum which was removed 31 minutes after the injection. This was the minimum period in which dye was found, but several other tests showed periods closely approaching this. Since it probably requires a few minutes before enough dye is accumulated to be visible, it was assumed that deposition takes place immediately following injection.

YOLK SECRETION

The large number of egg yolks utilized in this study made it possible to make a number of observations on the structure of the ovum and its manner of growth. Diagrams of the hen's ovum as shown in most texts on embryology do not indicate very accurately the manner of yolk accumulation. If a section is made bisecting the blastoderm, the latebra, and its neck throughout the length in an egg carrying dye rings at frequent intervals, a more accurate picture may be gained of the manner of deposition.

Because of the length and narrowness of the neck of the latebra it is difficult to secure a section through its entire length. Such a section as diagrammed in figure 2 shows that the successive layers of yolk do not form a right angle with the neck but tend in the outer portions of the yolk to follow the lines of the neck itself for a short distance. Figure 2 is a diagram showing the daily deposition of yolk as measured in a transverse section of the ovum. Frequently the dyed areas of the ovum show the region of the yolk mass contiguous to the neck of the latebra to be rather sharply depressed toward the center of the ovum (fig. 1, *C*). There seems to be considerable variability in the degree of depression of the layers of yolk, at the neck of the latebra, some ova showing very little. However, this may be due to the position of the transverse section, since some show the distortion much better than others. Immature ova do not show the depressed lines near the neck of the latebra, indicating that the layers of yolk as originally secreted slope upward along the neck and that the distortion develops at some time after deposition.

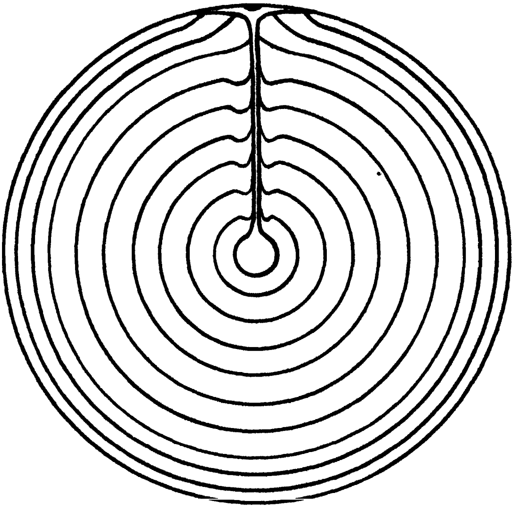

FIGURE 2.—Diagram of the yolk of an egg of hen No. 3925, giving the position of dye bands resulting from nine 24-hour-interval injections. Each concentric ring indicates the size of the yolk at the time of the injection. The latebra is in the center, and its neck joins the germinal disk.

Upon examining a yolk carrying dye rings throughout, one is impressed with the extreme uniformity of deposition. This fact made possible a study such as is here reported (fig. 1, *D*, *E*). The Regular spacing of dye rings secured by injections at uniform intervals shows the secretion of yolk to be both regular and uniform with respect to the surface of the ovum. It is also evident that there is very little flow or diffusion of materials within the yolk mass. Eggs kept for as long as 10 days had the dye bands as sharply defined as fresh eggs. This might be expected, since the dye is confined to the yolk globules and should diffuse very slowly through the water phase separating one globule from the next. The globules themselves, because of their size and close packing, would not be expected to move much except under pressure.

The uniformity of the layers of yolk leads one to speculate as to the nature of yolk secretion. Possible influencing factors are the composition of the blood stream in general, the vascular supply of the individual ova, and the permeability of the vitellin or follicular membranes. If the limiting factor were the surface over which secretion might occur one would expect the radius increments to remain uniform. As will be shown, this is not the case since the augmentations of the radius decrease rapidly as the ovum matures.

The studies also have shown that the actual volume of yolk secretion increases throughout growth except during the last 3 days. If the blood supply is the controlling factor we must assume that this improves as the ovum matures. Any such improvement would be in the vascular system of the particular follicle. It could not be due to changes in the composition of blood itself since ova in all stages of growth are being developed simultaneously.

In some eggs an interesting phenomenon was observed in connection with the manner of yolk secretion. Frequently the innermost dye bands were badly distorted. Such a condition is shown in figure 1, *B*. The fact that such distortions were not found in the bands of immature ova observed in one phase of this study, would seem to indicate that the inner bands were not laid down irregularly. It is possible that the distortions of the bands of yolk near the center may have resulted from excessive yolk deposition at some point or points in the follicular wall. If this continued for some time it would cause irregular pressure on the interior of the yolk and probably would result in shifting within the yolk mass. In such ova it was impossible to secure measurements of growth.

Observations indicated that although quantitatively yolk secretion was very uniform, there was evidence of qualitative variations. There were occasional conspicuous variations in yolk color. The most common qualitative difference appearing in the boiled yolks was a rubbery consistency of the interior instead of the characteristic granular nature. In the portion showing the rubbery consistency the dye bands were much diffused, indicating that the yolk material must have been much altered even before boiling. Riddle (*8*) mentions some yolks having a solid waxy interior; this is probably the condition found by the writers.

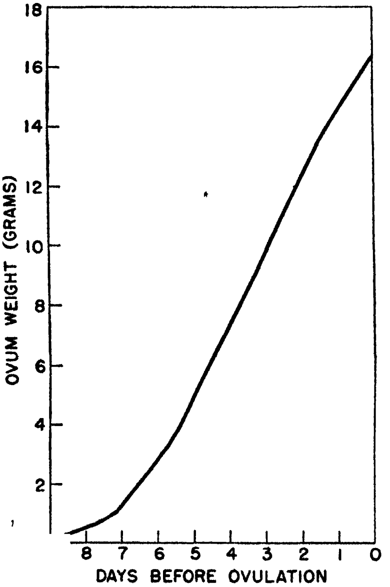

FIGURE 3.—Cumulative growth (weight in grams) curve constructed from the data on 367 yolks.

NORMAL GROWTH

The cumulative growth curve given in figure 3 is based on data from 38 birds, 367 eggs, and 1,169 dye bands. The records are from laying females of various ages, both White Leghorns and Rhode Island Reds. No significant growth differences were observed to be associated with age or breed.

The figure shows the growth in grams and time in days previous to ovulation. The actual measurements were recorded in units of the eyepiece micrometer at points of known distance from the surface of the ovum. To express the results in grams, the ovum was assumed to be a sphere (which is not exactly true), and the radius was calculated from the weight and specific gravity of the ovum. Then the measurements were converted to distances from the center, and the daily volume increments in grams were calculated from the density of boiled yolk, which was determined to be 1.027. The zero point on the horizontal scale is the time of ovulation, and the highest point in the curve is the yolk weight of the laid egg.

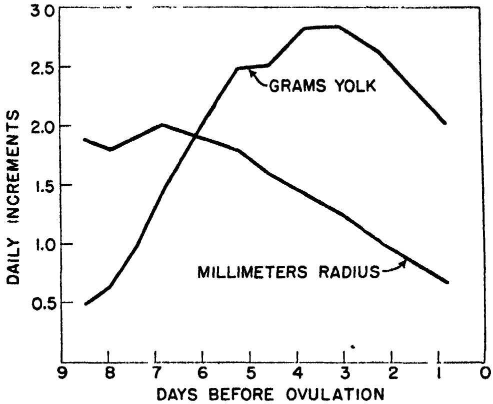

Figure 4.—Yolk growth curve showing the daily increments of radius and weight in 367 eggs.

It should be kept in mind that ovum growth as considered here includes only that period of relatively rapid increase in size occurring in the last 7 to 10 days before ovulation. Previous to that, growth is too slow to be measured by the technique here utilized. The curve indicates relatively slow yolk secretion for the first 2 days of the period (9 and 8 days before ovulation). Beginning the third day, the slope of the curve shows a higher rate of secretion, with evidence of a slight decline in the last 24 hours.

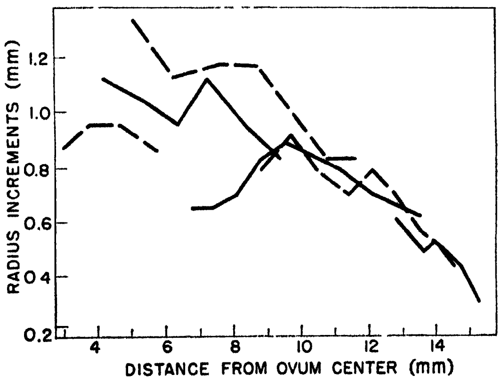

Figure 5.—Growth of individual ova of eggs laid by a single hen, measured in radius increments. The broken lines represent the growth of first eggs of a clutch and the unbroken lines that of successive eggs in a clutch.

A more definite picture of the rate of yolk secretion in the final cycle of rapid growth is shown in figure 4. Here growth is expressed as daily increments in grams of weight and millimeters of distance on the radius. It is seen from the figure that the growth when measured in units of the radius remains relatively constant until between 7 and 6 days before ovulation, when the ovum is about one-tenth its mature size. Then the daily radius augmentations are rapidly reduced.

It must be kept in mind that the surface of the ovum is increasing as it matures and even with constant increments of volume, the daily extension of the radius would decrease. For this reason a more enlightening measure of rate of yolk secretion is shown by the curve in figure 4, based on daily weight increments. This curve is of a form very different from that based on increments of the radius. The daily secretions of yolk material rapidly increase, reaching a somewhat flat maximum when the ovum has acquired about 60 percent of its size about 3 days before ovulation. During the last 48 hours there is a rapid decline in the rate of deposition of yolk material. So from the viewpoint of the amount of yolk materials secreted the efficiency of the follicle increases more or less progressively until the last few hours, when the rate of deposition declines rapidly.

Stieve (*13*) concluded from the examination of immature ova found in the hen's ovary that growth was very irrgeular. He believed that there was frequent alternation between rapid and slow daily growth. Since the curves in figure 4 are the expression of the mean growth rate of many ova, such alternations of growth rate would be obscured.

In figure 5 are shown the curves of 12-hour period growth rates of six individual successive ova of a hen. The growth curves of the ova of this particular hen are typical of those of a number of birds from which such data were obtained. Since the dye was injected for a limited period, growth data were available for only parts of most eggs. There are some irregularities, but there is no alternation of slow and rapid growth such as was described by Stieve. This fact is also brought out in figure 6. Some of the irregularities probably are due to distortions of the dye bands. There is evidence in figure 5 of some variation in growth rate among the ova of a hen, but in general the curves are in close agreement. Exceptional growth rates of individual ova of a hen were frequently noted in this study, indicating that factors operating within the follicle may have some influence on growth.

EARLY GROWTH

The data presented in figures 3 and 4 were not satisfactory for measuring accurately the initial and the final stages of growth of the ovum. Since the dye injections were made at 24-hour intervals any changes occurring in the last few hours previous to ovulation would not be detected. Another rather serious source of error was irregularities in the innermost bands of dyed yolk. The cause of these distortions was not determined, but when they occurred they were more evident in the bands near the center. In order to secure more accurate data on the initial and final stages of ovum growth a special technique was utilized. The term early growth is used here to apply only to the early stages of the final period of rapid growth. Other workers have shown that the ovum grows for months or years, but studies here reported include only the final stage, where growth is rapid enough to be measured by means of dye injections.

The growth near the center of the ovum was studied by a series of four 12-hour-interval injections of hens that were killed immediately after the last injection. This method had the disadvantage of securing immature ova, the mature sizes of which could not be determined, but it did give ova in which the dye rings were practically free of distortions. Since the ova thus secured were of varying size the measurements reading from the surface of the ovum, were not comparable. To overcome this difficulty the radius of each ovum was calculated from the weight and specific gravity. Then all measurements were converted into distances from the calculated center of the ovum. Ten laying females were killed after four injections, and measurements were made of growth in 12-hour periods. The latebra of the ovum is largely the ovum as it existed previous to the initiation of the final cycle of growth. The exact outline of the latebra is not well defined since the white latebra materials fuse gradually into the outer yellow yolk. In a hard-boiled yolk the latebra is of a soft, mushy consistency. The radius of the latebra is roughly 3 mm so the growth detectable by dye injections should start at approximately that distance from the center of the ovum. The smallest measurable dye ring had a radius of about 2 mm. The data from immature ova indicate that the early measurable growth is quite uniform, being near 1 mm in 12 hours. For 12-hour growth periods measured at 2, 3, 4, 5, 6, 7, and 8 mm from the center of the ovum; the mean increase in the radius was 0.91, 0.97, 0.96, 0.98, 0.97 0.92, and 0.99 mm, respectively. Inasmuch as the measurements were in units of the radius and the size of the sphere was constantly increasing, uniform increments would mean that the weight augmentations were increasing. Calculations of the growth in weight for the five periods were 0.10, 0.16, 0.26, 0.39, and 0.50 g, respectively. These figures were based on rather small numbers, four to seven band widths at a point, but since the remainder of the curve, taken on the same number of samples, is normal in shape, it seems that the results probably are dependable.

In most instances the measurements of growth of yolks from laid eggs were not considered accurate near the center. However, after the available data were studied, 11 eggs were selected with dye bands near the center which showed none of the irregularities frequently found it bands at this region. The mean growth rate was for a 24-hour period and was calculated from five to seven bands at each point. The growth recorded at points 3, 4, 5, and 6 mm from the center of the ovum were 1.30, 1.98, 1.82, and 1.73 mm, respectively, as measured on the radius. The foregoing data agree closely with those from immature ova since the latter were recorded for a 12- rather than a 24-hour period.

These results indicate that the initial growth rate of the ovum (first 48 hours) as measured by units of the radius is relatively uniform. The fact that the initial radius increments remain approximately constant means that the actual amount of yolk secretion must be increasing.

LATE STAGES OF GROWTH

The growth in the last few hours previous to ovulation is of interest since in some animals it has been proposed that a last-minute acceleration of growth might be responsible for rupture of the follicular membranes and for ovulation. The observations of Phillips and Warren (*6*) did not support this view, but the technique of the present experiment makes it possible to secure more accurate information on the final stages of ovum growth.

Since the routine injections of dye were made at 24-hour intervals, the data in figures 3 and 4 were not of much value in determining the rate of growth in the last few hours previous to ovulation. It has been shown by Warren and Scott (*14*) that ovulation takes place on an average one-half hour after the laying of the previous egg. Thus oviposition may be considered as a fairly accurate indication of ovulation time, but this holds only for eggs produced on successive days. Observation of a hen's laying record made it possible to predict that she would lay on the next 2 days. Such birds were injected with dye at from 1 to 15 hours previous to oviposition. The egg laid following injection was unaffected since it was in the oviduct at that time. However, since ovulation takes place almost immediately after oviposition of the previous egg, the period between injection and oviposition would measure the period of yolk deposition before ovulation. Since deposition of the dyed yolk starts immediately following injection, the distance between the inside of the dye ring and the surface of the egg would measure the amount of yolk materials secreted in the known period.

The question might be raised whether the use of dye injections measure the late-stage growth. If the final secretions were of a different nature they might fail to combine with the dye in the blood. However, observation indicated that this was not true since many eggs were found which carried a densely colored exterior.

Eighty-three hens were injected at varying short intervals previous to ovulation. When the egg resulting from ovulation following the injection was laid, measurements were taken of the growth of the ovum.

The data from this experiment are given in table 1 and are classified on the basis of the period of deposition. It should be kept in mind that the so-called period of deposition is the period intervening between injection with dye and the next oviposition. The ovulation which terminates deposition occurs at least a few minutes after oviposition so that the periods listed are somewhat less than those actually intervening. Inaccuracies of measurement are also a source of error of considerable consequence where very short periods of yolk deposition are involved. However, the results should not be vitiated as the comparison is of relative growth.

TABLE 1.—*Ovum growth in period immediately previous to ovulation*

Period of yolk deposition (hours)	Birds	Growth per hour		Period of yolk deposition (hours)	Birds	Growth per hour	
		Range	Mean			Range	Mean
	Number	*Millimeter*	*Millimeter*		*Number*	*Millimeter*	*Millimeter*
0.3 to 1.1	9		(1)	6.0 to 9.0	20	0.021-0.042	0.032
1.0 to 1.7	12		(2)	9.0 to 12.0	8	.034- .046	.038
1.0 to 3.0	4	0.015-0.036	0.025	12.0 to 15.0	17	.024- .057	.040
3.0 to 6.0	13	013- .061	.032				

[1] No dye.

[2] Trace.

In the eggs of nine females in which the period of yolk deposition ranged from 0.3 to 1.1 hours no dye could be detected. In 12 other females ovulating from 1.0 to 1.7 hours after injection the yolk showed such a slight trace of dye that it was not measurable. Four females having periods of deposition from 1 to 3 hours had measurable quantities of yolk deposited after the injection. In the egg of at least one female dye was detected when oviposition occurred just 1 hour after injection. Previous work at this station would indicate that ovulation might be delayed for as much as an hour after oviposition, although the average period is probably much less. This would mean that at least some yolk is secreted in the last 1 or 2 hours previous to ovulation.

From the data in table 1 there is fairly consistent evidence that the radius augmentations gradually become less and less as the ovulation time is approached. The evidence for this view as shown in table 1 is minimized by the fact that the longer periods included both the slower and more rapid growth stages. That is, the 12- to 15-hour group does not show just the rate of deposition in the 3-hour period between 12 and 15 hours before ovulation but for the whole period of 12 to 15 hours. If the stage of later growth could have been segregated from the earlier growth the differences in table 1 would probably have been greater. Owing to the small number of measurements available and the extent to which differences in yolk size would influence the results, no attempt was made to calculate the amount of yolk secreted in the periods considered. There is no evidence of any acceleration of the rate of deposition in the period immediately previous to ovulation, and it is probable that the retardation of secretion, as indicated in figure 4, continues to the time of ovulation.

YOLK SIZE

One of the problems of interest in the initiation of this study was the relationship of ovum growth to egg size. Other investigations at this station have indicated the variations in egg size of an individual hen may be influenced by fluctuations in yolk or ovum size. Therefore the cause of such variations is to be sought in the process of ovum growth. Differences in yolk size might be due to either or both the factors, rate and period of growth. The available data are not well adapted to measuring accurately the variations in period of growth, but they are suitable for comparisons of rate of growth.

Rate of growth as related to yolk size was measured by daily increments of the radii of the yolks, at varying distances from the yolk surface. In figure 4 it is seen that the daily radius increments decrease rapidly as the surface of the yolk is approached. Thus a comparison of the radius increments at the same distances from the outside of small and large yolks introduces a source of error. Such a point might be one-fourth of the way through a small egg and only one-fifth the way through a large one, thus making the two points not strictly comparable because of the decreasing growth rate nearer the surface. This source of error probably is not great but it cannot be ignored. However, this appeared to be the only manner in which growth could be compared.

In order to avoid the influence of other factors the birds of each injection experiment were arbitrarily divided into large and small yolk-size groups for comparison of rate of growth (radius increments). Although this limited the size range somewhat it was believed that comparing only the large- and small-yolk birds of the same injection experiment eliminated many variables such as breed, age, and season. Five injection experiments each including from 8 to 11 birds were studied. Growth in millimeters of the radii of the two groups in each experiment was compared at seven points ranging from 1 to 14 mm from the surface of the yolk. There were from 10 to 54 measurements for establishing a mean at each point in each size group. In the five-injection experiments 33 comparisons were made of the large- and small-yolk birds at various positions on the radius. In 18 of the 33 comparisons slightly slower growth was observed in the large-yolk groups. It appears probable from these studies that there is no difference in growth rate which may be considered a cause of yolk-size variation.

Since it is known that in the early period of a pullet's production her egg and yolk sizes rapidly increase, it is of interest to compare the growth rate of yolks of the same bird at different ages. Measurements were made of the growth rate of yolks of the very first eggs of eight White Leghorn pullets and again of yolks when the pullets were about 2 months older. In that period the mean yolk size had increased from 11.4 to 14.7 g, approximately a 29-percent increase. In table 2 is shown a comparison of growth rate of the yolks of these pullets at the two ages. Here again there was no consistent difference between the two sets of data. At three points the older birds had a slightly higher growth rate, and at six points the yolks of the younger birds grew more rapidly. If the 315 measurements of bands taken in the initial period are averaged they show a mean daily radius increment of 1.5 mm. The same birds when they were 2 months older and were laying larger eggs had a mean daily radius growth of 1.4 mm, calculated from 339 bands. The total number of measurements here do not agree with those in table 2 since some of those at extreme points were eliminated because the number of measurements was too small to give a dependable mean.

TABLE 2.—*Comparison of daily growth as measured on radius of the first eggs of a group of pullets with later and larger eggs of same individuals*

[Measurements were made at varying distances from the outside of the ovum]

Description of eggs	Distance from outside of ovum at which daily growth was measured	Measure ments	Mean growth	Description of eggs	Distance from outside of ovum at which daily growth was measured	Measure-ments	Mean growth
	Millimeters	*Number*	*Millimeters*		*Millimeters*	*Number*	*Millimeters*
First pullet eggs (mean weight 11.4 g)	2	34	0.88	Later eggs of same pullets (mean weight 14.7 g).	2	47	0.97
	3	40	1.16		3	36	1.18
	4	32	1.33		4	32	1.36
	5	32	1.50		5	26	1.46
	6	28	1.61		6	27	1.61
	7	28	1.78		7	28	1.75
	8	19	1.87		8	28	1.65
	9	34	1.79		9	30	1.65
	10	28	1.70		10	24	1.69
	11	20	1.68		11	19	1.59
Mean			1.50	Mean			1.40

It would seem from these results that differences in yolk size among individuals and the yolks of a single bird are probably not the result of more rapid growth. The results tend to show that for a given period the radius increments were the same in large and small yolks. Since it is admitted that large yolks have larger radii, the writers are forced to conclude that the growth must have continued for a longer period. It appears then that the data support only the view that some yolks are larger than others because they grow at the same rate for a longer period.

RATE OF LAYING

The data secured in this study should also throw some light on the physiological basis of rate of laying. It is known that differences in clutch (successive eggs) length are directly the result of interval varitions (period between ovipositions). Frequency of ovulation varies with the frequency of oviposition. The question arises: Is the more frequent rate of ovulation due to a higher rate of deposition of yolk materials? In order to answer this question the coefficient of correlation was calculated for the hen's mean interval (period between ovipositions) and the mean radius increase of her eggs for a 48-hour period. The mean growth rate measured by radius increase was secured by averaging the growth at a point near the middle of the radius. This avoided discrepancies resulting from differences in growth rate at different positions on the radius.

The coefficient of correlation between rate of yolk secretion in the ovum and period of egg formation (interval) was based on 108 eggs and had a value of -0.21 ± 0.04. The coefficient is very low and of questionable significance. The minus value would indicate that the birds that lay more frequently have a slightly more rapid yolk growth, but the correlation value is so low that at best, rate of ovum growth can have only a minor influence on rate of laying. With a given amount of yolk-building materials in the blood stream, the amount of secretion in any one follicle would depend on the number of developing follicles drawing on the supply. Therefore, it seems quite plausible that no close agreement would be found between rate of ovum growth and rate of laying. That is, a bird lays more rapidly because of having more ova growing simultaneously rather than because of an increased rate of secretion in a fixed number of follicles.

CLUTCH POSITION

The relationship of clutch position of the egg to its growth is of interest The laying of the first egg of the clutch is usually delayed 15 to 20 hours longer than the interval ordinarily intervening between successive eggs of a clutch. The interval between eggs is usually over 24 hours and results in the hen's laying a little later each day. When her schedule would cause a hen to lay late in the afternoon, she usually misses that day's production and starts a new clutch the next morning. Scott and Warren (*12*) have shown that the delay in oviposition of the first egg of the clutch is not to be accounted for by any additional time that the egg is in the oviduct but rather to a postponement of ovulation. This fact regarding the first egg of the clutch makes it of interest to compare the growth of the first and later eggs of a clutch with the view of gaining information on the mechanism responsible for the production of eggs in clutches.

In three experiments involving 26 birds and 270 eggs the measurements of growth were summarized to compare the first and later eggs of the clutch. The data were sufficient to construct reliable curves, which are shown in figure 6. The curves were constructed on the basis of growth at varying points measured from the surface of the yolk. The data are not well adapted to check on the time of initiation of or the period of growth, but they do show that yolks of first and later eggs in a clutch have a very similar rate of growth and form of growth curve.

The segregation of egg laying into clutches resulting from a delay of ovulation of the first egg of a clutch might be due to a number of factors. One possibility is that a slower growth rate of the yolk of the first egg of the clutch delays the time of ovulation. Another possible explanation is that the yolk of the first egg of the clutch grows normally but that the initiation of the late stage of rapid growth is delayed. If this explanation were correct, an examination of immature ova of a laying hen at any time should show an irregular gradation of the first egg of the clutch as compared with the preceding and succeeding egg. If the beginning of the development of the yolk of the first egg of the clutch were delayed, then at all stages of growth the difference between its size and that of the preceding egg (the last egg of the foregoing clutch) should be greater than the difference between it and the yolk of the succeeding egg which is a member of its own clutch. It would not be possible to make such a comparison from autopsies since clutch position could not be identified, but from the dye-injection data such comparisons were readily available.

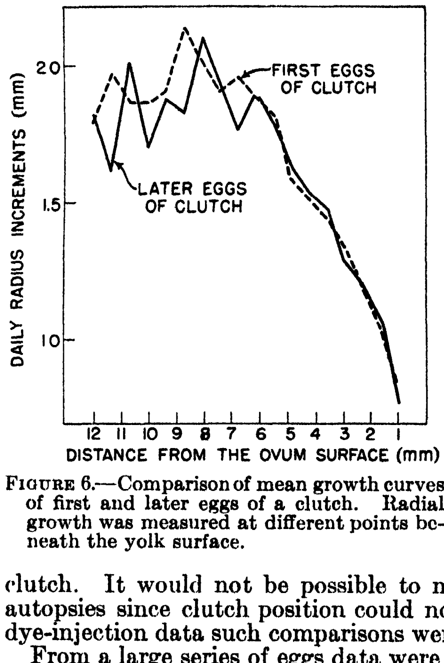

Figure 6.—Comparison of mean growth curves of first and later eggs of a clutch. Radial growth was measured at different points beneath the yolk surface.

From a large series of eggs data were summarized as to the relative sizes at various stages of growth of the yolk of the first egg of a clutch, that of the egg which preceded it, and that of the one which followed it. In all the eggs the distance of each dye band from the outside of the ovum was used as a measure of its size. So for any injection, eggs in the before-mentioned clutch positions could be compared in a single hen. Since measurements were made from the outside of the ovum there would be some error because of differences in yolk size. However, since the fluctuations of yolk size of a single hen are small, this source of error would be slight.

The size differences between the yolks of the first eggs of the clutch and the preceding and succeeding eggs at varying stages of growth were tabulated, there being 208 such comparisons. Statistical treatment showed the mean difference in radii between the yolk of the first egg of a clutch and the yolk of the last egg of the previous clutch to be 2.48±0.04 mm, while the difference in radii between the yolk of the same first egg of a clutch and that of the second member of its own clutch was 2.48±0.03. The results show that at all stages of growth there is maintained virtually the same size difference between the yolk of the first egg of the clutch and the last egg of the preceding clutch as there is between the first and second eggs of the same clutch. This would mean that the delay in ovulation of the first egg of a clutch is neither due to a delay in initiation of growth nor to a slower rate of growth.

This fact is further brought out in figure 7, which shows the cumulative growth curves of several eggs of the same hen. These curves are typical of a large body of data. As in figure 5 data were available for only parts of the curve for most ova. The slopes of the curves are very similar, indicating no differential growth rate between the ova of the first and later eggs of the clutch.

The distances between the curves of various ova are characteristically different throughout their growth. This indicates that not only did some ova start growing at a later period but that they maintained this difference throughout development. There was, however, no consistent relationship between these delays in the initiation of growth and clutch position.

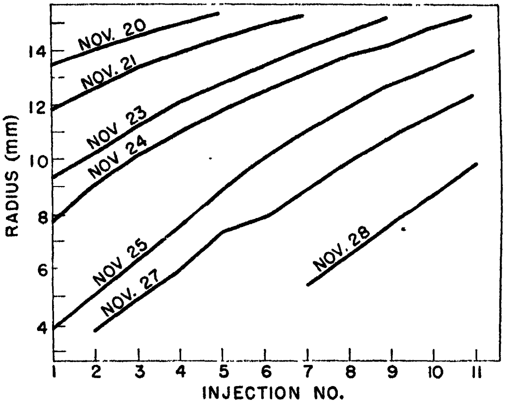

FIGURE 7.—Cumulative growth curves as indicated by radial length of individual ova of the eggs of a hen. The date of laying each egg is indicated on the curve. The points where the growth curves intersect the position of each injection indicate the relative growth rate of each ova of the series.

It seems that the mechanism which segregates eggs into clutches does not act on the ovum until near the time of ovulation, and the effect is that the yolk of the first egg of a clutch has a longer total growth period than that of the other eggs. This difference would amount to only 6 to 10 hours out of the approximate total of 200 hours that constitutes the final period of accelerated growth. Since this is a rather small difference when compared with the total time and since the rate of growth decreases as ovulation approaches, when the difference in time appears, one would not expect a large difference in size between the first and later yolks of a clutch.

One of the interesting problems in connection with growth of the ovum is the relation of this phenomenon to light. Studies by Warren and Scott (*14*) showed that light is the major causative agent for the tendency of the fowl to produce eggs in clutches. This fact was established by modifying the period of light. It was not determined just how light had its effect, but changes in the light conditions were expressed in the laying reactions of the bird in about 50 to 70 hours after the initiation of the change. This fact was arrived at from an experiment in which birds were subjected to a complete reversal of the periods of light and darkness. The birds responded by laying all of their eggs at night.

In one injection series the periods of daylight and darkness were reversed during the experiment for 14 White Leghorns. Thus ovum growth could be followed while the bird was adjusting itself to changed light conditions. It was hoped that the results might show how light acts normally in influencing the sequence of laying. The results were not very conclusive but seemed to show a retardation of about 10 percent in yolk secretion in the second 24-hour period after the change in lighting. The effects were greater in the less mature ova. Unless this result is due to the diminished food intake caused by the 24 hours of continuous darkness occurring at the time the change was made, no explanation presents itself.

YOLK FEEDING

Inasmuch as one of the factors controlling the rate of yolk formation must be the amount of yolk-forming materials carried in the blood, an experiment was conducted to determine the influence on ovum growth of feeding yolk material. Dye injections were made at 2 p. m. daily throughout the experiment. At the time of the fifth injection each of the 12 Rhode Island Red birds used was fed the yolks of two eggs and this was continued daily at the time of the six succeeding injections. Growth was measured in the yolks of all eggs produced as long as evidence of the dye injections was detectable in the eggs.

That the composition of the blood was profoundly affected by this feeding regime is shown by the fact that total fatty acids plus cholesterol in the blood was nearly trebled by the yolk feeding. Total fatty acids plus cholesterol was determined in whole blood by the method of Bloor as described by Hawk and Bergeim (*3, p. 442*). The average value for the group, which was 1,426 mg per 100 cc during the control period, rose to 3,964 during the period of yolk feeding.

TABLE 3.—*Ovum growth before and during the feeding of excessive amounts of yolk, the yolk feeding starting at the fifth dye-injection period*

Injection No.	Growth records	Mean radius increments in 24 hours following injection	Injection No.	Growth records	Mean radius increments in 24 hours following injection	Injection No.	Growth records	Mean radius increments in 24 hours following injection
	Number	*Millimeters*		*Number*	*Millimeters*		*Number*	*Millimeters*
1	43	1.69	5	37	1.68	8	34	1.81
2	40	1.57	6	31	1.71	9	33	1.84
3	30	1.60	7	33	1.84	10	34	1.76
4	35	1.77						

The growth from the time of one injection of dye to the time of the one which followed is given for the entire series in table 3. Growth was measured in millimeters of the radius. Each 24-hour growth period was measured by from 31 to 43 samples; adequate sampling from all parts of the ovum was thus provided, and the mean is dependable. The ovum growth following the first four dye injections was made under normal feeding conditions, the birds receiving the standard Kansas State College laying ration. From the fifth injection on, the females received the two egg yolks per day in addition to the usual ration. It is not known exactly how much time would be required for the additional yolk feeding to have its effect on yolk deposition. It seems improbable that much effect could be noted in the 24 hours following the first feeding, and this period was disregarded in the comparison of growth rate before and after yolk feeding. The mean of the 157 24-hour radius increments previous to fat feeding was 1.67 ±0.028 mm, while the 165 increments after the fat was fed averaged 1.83 ±0.050 mm. Although the rate of growth was increased almost 10 percent in the period of yolk feeding, the difference is of doubtful statistical significance. In each 24-hour period after the sixth injection, growth was greater than the mean of the periods before yolk feeding.

It would appear therefore that additions of yolk fat to the ration may accelerate ovum growth. This stimulation might be expressed either in an earlier ovulation or a larger egg yolk. The weight of yolks of eggs laid during the first 4 days of the experiment were compared with those laid after the second day of fat feeding. The 29 yolks of eggs laid in the period before fat was fed had a mean weight of 17.75 ±1 10 g while the 68 laid during the fat-feeding period weighed 18.29 ±1.21 g. Although the yolks during yolk feeding were larger than those in eggs laid before, the increase was not enough to be considered statistically significant.

In order to determine whether the slight difference was really significant, a more extensive experiment was undertaken. Twenty-four White Leghorn pullets were used and were fed two yolks each for 10 days. The yolks of 98 eggs produced in the 5-day period just previous to yolk feeding were weighed individually. Beginning on the fifth day of yolk feeding, the yolks were again weighed for an 8-day period, in which 158 eggs were produced. Eggs were saved a little beyond the yolk-feeding period since yolk growth is completed at ovulation, which is 24 hours before laying and some time would be required for the assimilation of the additional fat that was fed. There is a possibility of a change in yolk size normally occurring in the birds during the period of the experiment as a result of age, temperature, or other uncontrolled factors. In order to eliminate errors from such a source, yolks were again weighed in a second control period following yolk feeding. Eggs were not saved until 6 days after the yolk feeding had been stopped. The control period following yolk feeding lasted 5 days; during that time 100 yolks were weighed. A recording thermometer was used, and the temperature during the yolk-feeding and during the two control periods was found not to vary appreciably.

The mean weights of the yolks produced during the yolk-feeding period was greater than that of those produced during either the preceding or succeeding control periods. The weight of the 158 yolks produced during the yolk-feeding period was 16.56±0.074 g, whereas the 98 yolks produced immediately before weighed 16.03±0.094 g. The 100 yolks produced in the control period following the yolk feeding weighed 16.21±0.107 g. If the results during the two control periods are combined, the mean yolk weight is 16.12±0.071 g. The difference between the two control periods was not statistically significant, but the yolk weights during the yolk-feeding period were significantly greater than the weight of those produced in the control periods, the actual difference being more than four times the probable error of the difference. These results do not prove that the fat content of the blood is the limiting factor in yolk growth, but they indicate that rate of growth and yolk size may be increased by addition of yolk material to the diet.

KAMALA EFFECTS

Maw (*5*) has shown that the administration of kamala as a vermicide not only causes a decline in rate of production but also results in a decrease of egg size. Since variations in the whole egg size are frequently accompanied by changes in yolk size, kamala was fed to determine how it affected yolk growth. During the course of a dye-injection series 1 g of kamala was fed to each of four birds. Beginning about 24 hours after the administration of kamala, the yolk deposition not only was much retarded but it seemed to be of abnormal consistency. The dye rings were not sharply differentiated; this indicated a disruption of the secretory processes. It would, therefore, seem that kamala disturbs and checks the process of yolk secretion and so affects egg size.

SUMMARY

The technique of injection of the fat-soluble dye, Sudan III, into the hen's blood stream at uniform intervals was utilized for studies of growth of the ovum. Microscopic measurements of the daily radius increments from the concentric dye rings on the cut surface of boiled yolks made it possible to record the growth of the ovum. The period studied was the last 7 to 10 days before ovulation, in which growth is greatly accelerated.

When growth was measured in radius increments it was found to be relatively constant from the ninth to the seventh day before ovulation and then to decrease rapidly thereafter. If the actual daily secretion of yolk in grams is considered, it increases rapidly from the ninth to the fourth day before ovulation and after the third day before ovulation it decreases rapidly. Some growth was detectable within an hour of ovulation.

Yolk-size variations among hens and among an individual hen's eggs were not found to be associated with different growth rates of ova. This would indicate that variations in yolk size are to be accounted for by differences in the period of growth rather than by the rate of growth.

Differences in rate of laying as measured by the interval between ovipositions were not found to be closely associated with variations in rate of ovum growth.

The break between clutches (consecutive eggs) which is accompanied by a 15- to 18-hour delay in ovulation of the first egg of the clutch was studied in relation to ovum growth. No evidence was found of either a slower rate of growth or any delay in the initiation of growth. This would indicate that the first egg of the clutch may continue its growth longer than successive eggs of a clutch.

Feeding excessive amounts of egg yolk slightly increased both the rate of yolk secretion and the size of the mature ovum. Feeding kamala seemed to disrupt and retard yolk secretion.

The results of this study seem to indicate that variations in yolk size and the rate of ovulation are not conditioned by rate of ovum growth. The physiological basis of these phenomena is probably associated with the factors controlling the initiation of the final stage of accelerated growth.

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ADEQUACY OF LABELING OF CERTAIN TEXTILE FABRICS WITH REGARD TO FIBER CONTENT[1]

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INTRODUCTION

It has become difficult to ascertain the fiber content of fabrics by their appearance and handle or by chemical and burning tests. Formerly, it was a simple matter to determine whether a fabric was silk, cotton, linen, or rayon. New finishes, however, have been devised to give the synthetic fabrics the appearance of wool, linen, or silk. Furthermore, there has been an increasingly large production of fabrics composed of two or more kinds of fibers. Experienced textile workers have frequently been unable to determine fiber content without using exacting methods of identification. Because the kinds of fibers used cannot be determined without reliable laboratory techniques, fabrics on the market should have labels stating accurately the fiber content. Such accurate statements of the fiber content would enable the consumer to exercise proper care in dry cleaning and laundering and would be of particular value to those who are allergic to certain textiles (*18*).[3]

This paper reports an investigation to ascertain to what extent authentic information was available to the purchaser concerning the fiber content of fabrics on the open market.

RULINGS ON FIBER CONTENT OF TEXTILES

Some attempts have been made to state the fiber content of textiles. The Federal Trade Commission adopted a ruling on May 26, 1930, that "the word 'wool' shall not be used in any way in labeling, advertising, merchandising, or selling of knit underwear unless the percentage by weight of wool contained in the garment be stated."[4] A commercial standard for wool and part-wool blankets (*23*) became effective April 1, 1933, which provided for the labeling of part-wool blankets with the guaranteed minimum wool content, and one for wool and part-wool fabrics (*24*) became effective January 1, 1938. On October 27, 1937, the Federal Trade Commission issued its ruling of fair trade practices for the rayon industry (*20*). The new rules define rayon as "the generic term for manufactured textile fiber or yarn produced chemically from cellulose or with a cellulose base." In mixed fabrics containing rayon, it is required that the kinds of fibers and the percentages of each by weight be given on the labels. The ruling concerning rayon has met with some opposition. New rules were pro-

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[2] Contributions by the junior writer were based on results reported in a master's thesis, Adequacy of Labeling of Certain Mixed Fabrics. On file, library, Kans. State Col. Agr. and Appl. Sci.

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mulgated November 4, 1938, for the silk industry. These rules recommend that silk fabrics be labeled as such and that mixed fabrics containing silk have the percentages of the different fibers given (*21*). The Federal Trade Commission also proposed trade practice rules for the wool industry in November 1938 (*22*).

MATERIALS

The fabrics tested in this investigation were purchased in 18 different stores in 7 towns and cities of the Midwest. At the time of purchase any information on the labels with regard to fiber content was noted. When the fiber content was not stated on the label, as much information as possible was obtained from the salesman. Of the 268 fabrics purchased, 133 contained only one kind of fiber, and 135 were of mixed fiber content. It was soon learned that the information concerning mixed fabrics and synthentic fabrics was least reliable, and an effort was made to select as wide a variety of these as possible. Few all-cotton and all-linen fabrics which were thought to be correctly labeled were purchased.

METHODS OF IDENTIFICATION AND ANALYSIS

MICROSCOPIC IDENTIFICATION OF FIBERS

Before any quantitative determinations of the fabrics were made, the kinds of fibers were determined. The microscope was generally used for identification because microdeterminations required less time and proved more conclusive than chemical tests. A comparison of longitudinal and cross-sectional mounts of the unknown fiber with a series of known specimens was employed. A permanent set of slides of authentic fibers most likely to occur in textiles was prepared. Plates 1 and 2 show photomicrographs of three rayons.

Many fibers could be identified by longitudinal mounts, but for the identification of silk and the various rayons cross sections were necessary. Cross-sectional mounts, accordingly, were made by the Viviani cork method (*5*, *14*, *15*, *25*).

Surface details of the wool fibers were studied by means of casts (*3*, *8*, *9*, *15*).

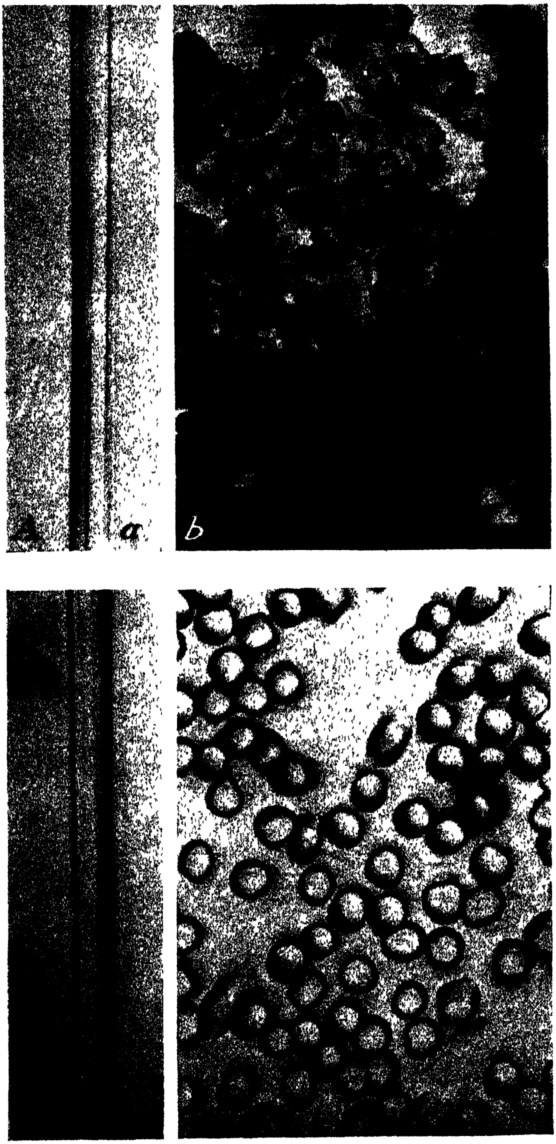
Polarized light proved to be valuable in the identification of highly birefringent vegetable and rayon fibers. Difficulty in differentiating between linen and mercerized cotton fibers was overcome by examination between crossed Nicol prisms. Polarized light brought the nodes of linen into greater prominence.

In some of the identification work, confirmatory tests were made by using certain dyes. Millon's reagent was used in distinguishing animal from vegetable fibers, and Neocarmin B and Meocarmin W were used in identifying the rayons.

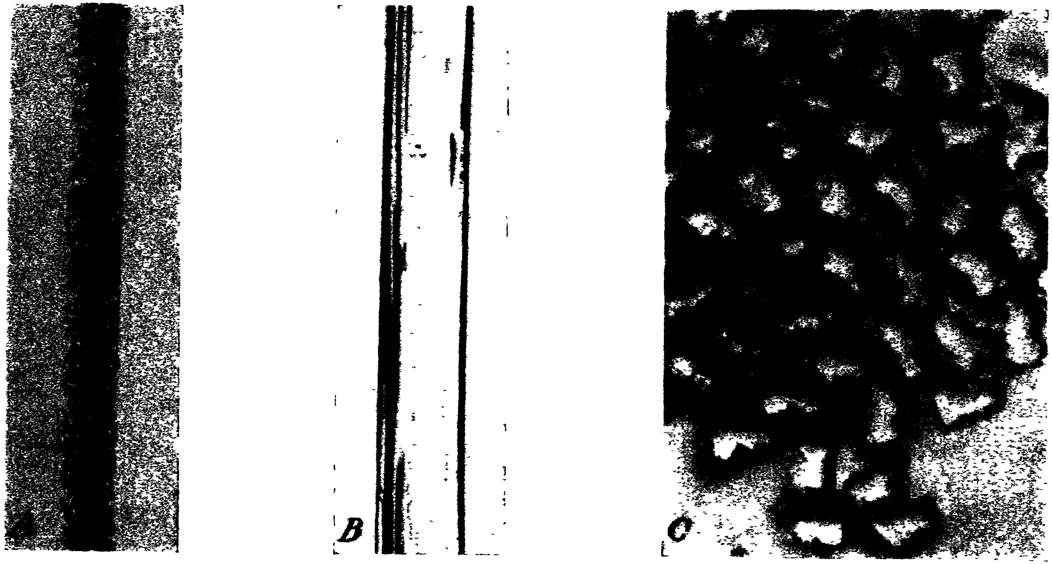
QUANTITATIVE MECHANICAL ANALYSIS OF MIXED FABRICS

When a fabric was composed of one kind of fiber, only the fiber identification was needed. When a fabric contained two or more kinds of fibers, a quantitative analysis was necessary. This analysis was made as follows:

Two samples of each fabric, weighing approximately 5 g were taken. The sizing, finishing, and other nonfibrous materials were



A, Cellulose acetate rayon fibers: *a*, Longitudinal view, Canada balsam mount, × 380; *b*, cross section, air mount, × 350. *B*, Cuprammonium rayon fibers: *a*, Longitudinal view; *b*, cross section. Glycerin mounts, × 380.



Viscose rayon fibers: *A*, Longitudinal view, delustered, Canada balsam mount, × 350 *B*, longitudinal view, lustrous, glycerin mount, × 380: *C*, cross section, lustrous, glycerin mount, × 380.

removed by extraction with carbon tetrachloride and a 3- to 5-percent aqueous solution of a starch-hydrolyzing enzyme. These operations were carried out according to the directions of the American Society for Testing Materials (*1*). The dried weight of the samples was found, and a suitable quantitative analysis was used in determining the fiber content.

There are three fundamental ways of determining the fiber content quantitatively– mechanically, microscopically, and chemically. The mechanical method when applicable is the most accurate. When the fibers are mixed before the yarn is spun, mechanical separation is not feasible. Ply yarns having one ply of one kind of fiber and one of another are often used in weaving fabrics. The mechanical separation of the plies is frequently a better method to use than a tedious and less accurate chemical analysis. Fabrics woven from two different kinds of yarn are easily analyzed mechanically. Many fabrics have the warp yarns of one fiber and the filling of another.

QUANTITATIVE CHEMICAL ANALYSIS OF MIXED FABRICS

Several chemical analyses were checked; samples of known fiber were used in order to determine which methods were most applicable. If cellulose acetate rayon was present in a mixed fabric, it was removed first by dissolution in acetone. Acetone readily dissolved acetate rayon with an error of less than 1 percent whenever cotton, silk, wool, or regenerated cellulose was present. The method followed in the use of acetone was that given in the Standards on Textile Materials (*1*). Two samples were run simultaneously and agitated at room temperature in the Launder-Ometer for 15 minutes in about 50 times their weight in acetone.

Silk, if present, was removed next. The method of dissolving silk in Lowe's reagent [5] at room temperature for 20 to 30 minutes was found to be the quickest and most accurate of those tried. The residual samples always gained weight because of the deposit of a gelatinous copper compound which was removed by rinsing the samples in a 1- to 2-percent hydrochloric acid solution before washing with distilled water.

There are several methods (*1, 2, 6, 7, 10, 12, 13, 19, 24*) of determining the wool content in mixtures of wool, cotton, and rayon. Cotton and regenerated cellulose may be separated from wool by carbonization with aluminum chloride. In the work reported herein, the sample was immersed for 10 minutes in a boiling solution of aluminum chloride containing 9 g of hydrated chloride per 100 ml of water. After the sample was heated in an oven at 105° to 110° C. for 2 hours, it was rubbed against a 100-mesh screen with sufficient pressure to powder the carbonized cellulose and pass it through the screen. The material was passed through the screen again to recover any wool fibers that had passed through the first time. The wool was agitated with about 100 ml of a 10-percent solution of hydrochloric acid, then washed with distilled water until free from chlorides and dried. The error involved in this method was less than 1 percent.

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[5] The reagent is made of 16 g of copper sulphate in 150 ml of water, with the addition of 10 g of glycerol. A concentrated solution of sodium hydroxide is added until the precipitate of copper hydrate, which at first forms, dissolves.

When wool comprised the greater portion in a mixture of wool and cotton or wool and regenerated cellulose rayon, it was removed with a solution of potassium hydroxide as shown by Mease and Jessup (*12*). The samples were boiled for 10 minutes in a 5-percent aqueous solution of potassium hydroxide. A reflux condenser was used to prevent concentration of the alkali because of evaporation. The samples were rinsed in a 5-percent aqueous solution of acetic acid, and then in distilled water until free from acetic acid. Potassium hydroxide dissolved wool readily with little effect on cotton. Regenerated cellulose was affected somewhat by this method, the error being approximately 3 percent; but with cotton the error was less than 1 percent.

Cotton was separated from regenerated cellulose by an aqueous solution of calcium thiocyanate as described by the American Society for Testing Materials (*1*). The samples were agitated for 1 hour at 70° C. in 200 ml of calcium thiocyanate, of specific gravity 1.35 to 1.36, slightly acidulated with acetic acid. The error was approximately 2 percent.

Table 1 shows the effects of the various reagents upon the textile fibers. Correction factors were not used except when potassium hydroxide was used to dissolve wool mixed with regenerated cellulose rayon. The weight of regenerated cellulose was found by multiplying the dry weight obtained in the analysis by 1.03.

TABLE 1.—*The effect of various solvents on different textile fibers*

Fiber	Acetone	Lowe's reagent	Aluminum chloride	Potassium hydroxide	Calcium thiocyanate
Cellulose acetate.	All dissolved				
Silk	Less than 1 percent dissolved	All dissolved			
Cotton	do	Less than 1 percent dissolved.	All carbonized	Less than 1 percent dissolved	Approximately 2 percent dissolved
Regenerated cellulose.	do	do	do	Approximately 3 percent dissolved.	All dissolved.
Wool	do	do	Less than 1 percent carbonized.	All dissolved	Approximately 2 percent dissolved.

QUANTITATIVE MICROSCOPICAL ANALYSIS OF MIXED FABRICS

Although there is an increasing realization that the volume of each fiber present in a mixed fabric is as important as the weight of each fiber present, comparatively little work has been done on quantitative microscopical analysis. Heretofore, most quantitative analyses of fibers have been based on percentage weight as determined by chemical analysis. The microscope lends itself to volumetric as well as gravimetric determinations. Quantitative microscopical analysis of textile fabrics is often the most desirable, and sometimes the only, method which can be used. Fabrics made of yarns which are blends of fibers lend themselves to microscopical analysis. If one kind of fiber constitutes a very small percentage, it is often better to resort to microscopical analysis rather than to a chemical analysis in which the loss of the fiber of small percentage may amount to a great deal.

Blends of viscose rayon and linen, or of viscose rayon and cotton may be analyzed by dissolving the rayon in calcium thiocyanate. After the solution is used, it must be diluted until the rayon is precipitated, and then evaporated before it is ready to be used again. The microscopical analysis is often to be preferred to the chemical analysis.

Blends of different kinds of wools cannot be separated by chemical analysis, and microscopical methods must be used. Skinkle (*16, 17*) and von Bergen (*26*) made analyses of wool and mohair and other animal fibers, identifying the kind of fiber by measuring scale sizes and diameters and counting the number of each kind in a cross section of yarn.

Heermann and Herzog (*4*) showed that the percentage of each kind of fiber in a mixture could be found by the equation

$$\text{Percentage of fiber } A = \frac{100 n_a g_a}{n_a g_a + n_b g_b}$$

where n is the number of fibers and g is the weight of each fiber per unit of length. Skinkle (*17*) in his work on animal fibers, which are nearly round in cross section and of approximately the same density, expressed the percentage by the equation

$$\text{Percentage of fiber } A = \frac{100 n_a d_a^2}{n_a d_a^2 + n_b d_b^2}$$

where d is the average diameter of the fibers and n is the number of fibers.

This equation can be modified to calculate the percentage of fibers which are of irregular cross section without the determination of weight of the fibers per unit length as done by Heermann and Herzog.

Two methods were used by the present writers for counting the number of each kind of fiber. For blends of linen and viscose rayon, of cotton and viscose rayon, or of cotton and linen, the number of each kind of fiber was counted by cutting as small a length of the yarn as possible, less than one-sixteenth of an inch, with small shears. The small lengths were mounted in glycerin and teased out as uniformly as possible on the slide. A magnification of 100 or less was used with a 16-mm objective, and the total number of fibers of each on the entire slide was counted by moving the slide with a mechanical stage which had two graduated scales at right angles. The distinguishing of linen from cotton could often be facilitated by placing the slide between crossed Nicol prisms. Ten slides or more were prepared from yarns taken from various parts of the fabric, and the average of the counts on the 10 slides was taken as the number of fibers of each kind in the yarn.

The above method was used for blends in which one kind of fiber made up a very small percentage of the yarn and for fibers of which it is difficult to make cross sections.

For blends of different kinds of wools, of rayon and wool, and of wool and cotton, the counting of the fibers was done by making cross sections by the Viviani cork method. A magnification of about 300 with a 4-mm objective was used. A field was chosen at random, and the number of sections of each kind of fiber was counted. Ten to twenty different fields of the same size were counted, and the average of each kind was taken as the number of fibers. Plate 3 shows photomicrographs of cross sections of yarns of various kinds of mixed fabrics.

The relative areas of the fibers were found by drawing 10 or more cross sections with the camera lucida as pointed out by Preston (*14*). The cross sections were cut out and weighed on an analytical balance, and the average weight of the paper drawings of each kind of fiber was calculated and taken as proportional to the size of the fiber. The percentage by volume of each kind of fiber in a blend can be expressed by the equation

$$\text{Percentage of fiber } A=\frac{100n_aw_a}{n_aw_a+n_bw_b}.$$

The percentage by weight of each can be expressed by the equation

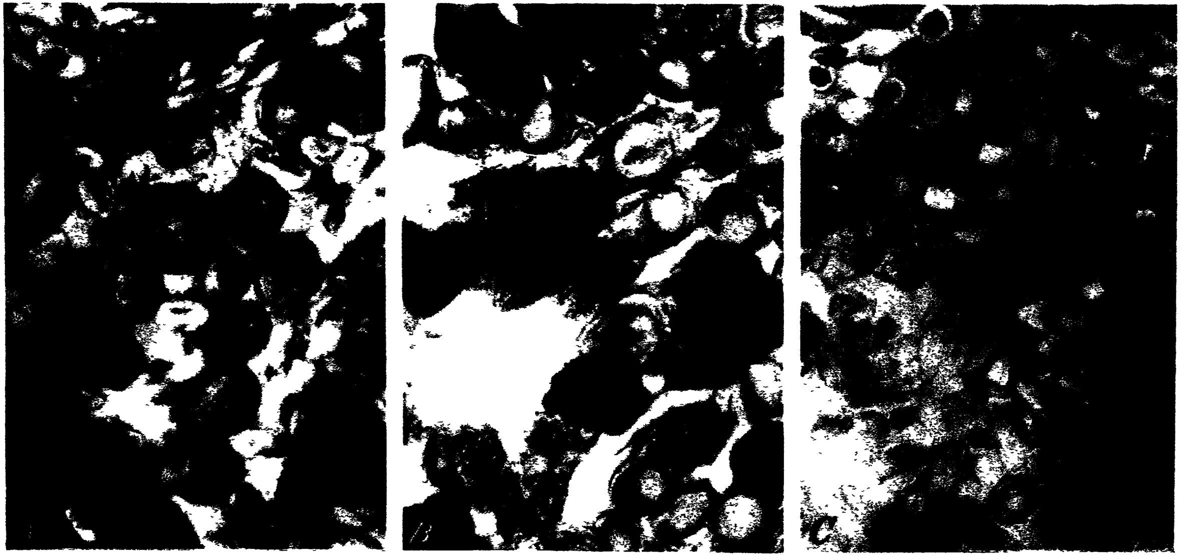
$$\text{Percentage of fiber } A=\frac{100n_aw_ag_a}{n_aw_ag_a+n_bw_bg_b}.$$

In the equations n is the number of fibers, w is the average weight of camera-lucida drawings of the cross sections, and g is the specific gravity of the fiber. The values of the specific gravities were taken from Heermann and Herzog (*4*) and the International Critical Tables (*11*).

RESULTS AND DISCUSSION

Table 2 gives the results of the examination of the 133 fabrics of one kind of fiber. In each case the information on the label and that given by the clerk were compared with determinations made in the laboratory. Ordinarily the salesmen gave no further information if the fiber content of the textile was stated on the label. An acetate rayon fabric was considered accurately labeled if given as celanese rayon, acetate rayon, or acetate, and only partially accurate if given as rayon or celanese. The information with regard to fiber content of a fabric of cuprammonium rayon was considered accurate if given as cuprammonium rayon, cuprammonium, or Bemberg rayon, and partially accurate if given as Bemberg or rayon. In the case of viscose rayon, viscose rayon or viscose was counted as accurate, and rayon as partially accurate.

A mixed fabric was considered to be accurately labeled if the kinds of fibers that it contained were given. Viscose rayon had to be given as viscose rayon or viscose; cuprammonium rayon as cuprammonium rayon, cuprammonium, or Bemberg rayon; and acetate rayon as acetate rayon, acetate, or celanese rayon. If the percentage of fiber content was stated, the information was considered accurate if the percentage was correct within 10 percent. It was considered partially accurate if one kind of fiber contained in the fabric was given, and the others were not. Table 2 also gives the results of the examination of these fabrics.



A, Blend of wool and cotton, cross section, glycerin mount, × 380. *B*, blend of wool, delustered viscose, and dyed viscose, cross section, glycerin mount, × 380. blend of wool, silk, and rabbit fur, cross section, glycerin mount, × 380.

TABLE 2.—*Adequacy of information secured from labels or salesmen concerning fabrics composed of one kind of fiber or of mixed fibers*

FABRICS OF ONE KIND OF FIBER

Fabrics analyzed		Fabrics that were –				Fabrics regarding which salesmen gave –			
Kind	Number	Labeled	Labeled accurately	Labeled partly accurately	Labeled inaccurately	Information	Accurate information	Partly accurate information	Inaccurate information
		Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Cotton	4	2	2	0	0	2	1	1	0
Linen	3	3	3	0	0	0	0	0	0
Silk	26	11	11	0	0	16	13	2	1
Wool	14	5	5	0	0	9	8	0	1
Acetate rayon	16	7	1	5	1	9	2	7	0
Cuprammonium rayon	13	5	1	4	0	8	1	7	0
Viscose rayon	57	24	0	23	1	35	0	31	4
Total	133	57	23	32	2	79	25	48	6
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Proportion	--	100	40.4	56.1	3.5	100	31.6	60.8	7.6

FABRICS OF TWO OR MORE KINDS OF FIBERS

Kind	Number	Labeled	Labeled accurately	Labeled partly accurately	Labeled inaccurately	Information	Accurate information	Partly accurate information	Inaccurate information
		Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Cotton and wool	11	2	0	2	0	11	4	5	2
Cotton and viscose	15	3	0	3	0	13	0	11	2
Cotton and linen	6	5	0	5	0	2	0	2	0
Wool and rabbit fur	3	0	0	0	0	3	1	2	0
Wool and viscose	21	4	0	4	0	19	0	18	1
Wool and silk	5	2	0	2	0	5	1	4	0
Silk and viscose	7	2	0	2	0	5	0	5	0
Acetate and viscose	43	12	0	12	0	31	0	30	1
Various fabrics composed of 2 kinds of fibers	6	1	0	1	0	5	2	3	0
Various fabrics composed of 3 kinds of fibers	18	3	0	3	0	16	1	14	1
Total	135	34	0	34	0	110	9	94	7
		Percent		*Percent*		*Percent*	*Percent*	*Percent*	*Percent*
Proportion	--	100	--	100	-	100	8.2	85.4	6.4

SUMMARY

Table 2 shows that of the fabrics analyzed those made of cotton, linen, silk, and wool were commonly accurately labeled and that salesmen, in the main, gave accurate information with regard to these fabrics bearing no label with regard to fiber content. Of all the fabrics made of one kind of fiber, the information concerning the three types of rayon was found to be least accurate. These fabrics were often represented as "rayon," but they were usually not designated as acetate, viscose, or cuprammonium rayon. The word "celanese" was often used instead of acetate; Bemberg and Bemberg rayon were always used instead of cuprammonium rayon; and rayon was used instead of viscose rayon. Of the 133 fabrics of 1 kind of fiber analyzed, 57 were labeled. Of these, 40.4 percent were accurate; of the 79 for which salesmen gave information, 31.6 percent were accurate. Thus the information on the label was found to be more accurate than that given by the salesmen.

Of the 135 mixed fabrics, 117 were made of 2 kinds of fibers, and 18 were of 3 kinds. Many of these had warp yarns of one kind and filling of another. Some were made of yarns which were blends, and others of ply yarns composed of plies of different kinds of fibers. Cellulose acetate rayon and viscose rayon were most frequently found in combination. Only one fabric of each of the following combinations was found: Viscose and linen, linen and wool, acetate and cuprammonium rayons, acetate rayon and silk, wool and cuprammonium rayon, and wool and mohair.

Little information was obtained concerning the percentage of fiber content, either from the labels or from the salesmen. Only one fabric bore a label stating the percentage of fiber present. The label stated that the fabric contained 25 percent of wool, but analysis showed only 15 percent to be present. The clerks gave percentages for fiber content for 12 fabrics, of which 1 was accurate, 10 partially accurate, and 1 wrong.

Of the 135 mixed fabrics examined, only 34 were labeled; and all of these were only partially accurate. The clerks gave information concerning 110, of which 8.2 percent were accurate, 85.4 percent partially accurate, and 6.4 percent wrong.

It is apparent that more of the fabrics of one fiber are labeled, and more of the information accurate, than of mixed fabrics, but in case of the labeled fabrics of one kind of fiber the accuracy was only 40.4 percent. The accuracy of the information, from labels and from salesmen, for both groups of fabrics was inadequate.

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A PHYSIOLOGICAL STUDY OF THE PREFILLING PERIOD OF FRUIT DEVELOPMENT IN THE PECAN[1]

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INTRODUCTION

In a report of the study of fruit development in the pecan (*Carya pecan* Engl. and Graebn.[2]) made in 1932,[3] the authors pointed out that lack of material prevented the collection of samples as frequently as was desired during the prefilling period. In a similar study made during the spring and summer of 1933 this condition was corrected by taking samples of Burkett nuts at least every 2 weeks from May 18 (the first sampling date in 1932) to August 28. Since the 1933 crop developed a little earlier than that of 1932, the nuts of the first samples were somewhat larger, and those sampled on August 28 were considerably further advanced than those for corresponding samples taken in 1932.

SAMPLING AND METHODS

During the first part of the experiment samples were taken every 2 weeks, but beginning August 1 they were taken each week in order to follow more closely the rather rapid process of shell formation. On August 14 and thereafter, separations were made between shuck and shell and samples of the two parts were preserved for analysis. The stages of development of the nuts on the various sampling dates are given in table 1.

TABLE 1.—*Stage of development of pecan fruits on the dates of sampling*

Sampling date	Weeks after full bloom	Average green weight per fruit	Stage of development
1933	*Number*	*Grams*	
May 18	6	0.22	All fruits apparently alike except that they increased in size as the season advanced.
June 1	8	.55	
June 15	10	1.54	
June 29	12	3.04	
July 14	14	6.89	
Aug. 1	16	16.53	Shell just beginning to harden at blossom end. Ovule had grown quite a distance into the white spongy material. Seed coat filled with clear colorless fluid or endosperm.
Aug 7	17	19.85	Shell quite hard from apex to about half way to basal end of nut. Considerable layer of white spongy material between seed coat and shell. Ovule filled with clear colorless fluid of low viscosity.
Aug. 14	18	23.29	All of the shell sufficiently hard to permit removal of shuck by paring and scraping. Still some white material between seed coat and shell. Endosperm same as before
Aug 21	19	27.68	Whole shell quite hard. Cotyledons beginning to line seed coat. Endosperm more viscous than before Spongy material still white except near bundles, where it was light brown.
Aug. 28	20	28.25	Cotyledons beginning to thicken. Endosperm mostly absorbed.

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[1] Received for publication October 24, 1938.

[2] Synonym, *Hicoria pecan* (Marsh.) Britton.

[3] THOR, CLIFFORD J. B., and SMITH, CHARLES L. A PHYSIOLOGICAL STUDY OF SEASONAL CHANGES IN THE COMPOSITION OF THE PECAN DURING FRUIT DEVELOPMENT. Jour. Agr. Research 50. 97-121, illus. 1935.

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The sampling and analytical methods used were essentially the same as those employed for the 1932 material.[4]

ANALYTICAL DATA

The analytical data are summarized in table 2.

TABLE 2.—*Composition of pecan fruits throughout the prefilling period, 1933*

Part of fruit	Date of sampling	Dry matter	Constituents of dry matter				Weight per fruit or part					Ratio of total sugar to moisture
			Total nitrogen	Ash	Total sugar	Acid-hydrolyzable polysaccharides	Dry matter	Total nitrogen	Ash	Total sugar	Acid-hydrolyzable polysaccharides	
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Grams*	*Gram*	*Gram*	*Gram*	*Grams*	
Whole fruit	May 18	31 66	1 76	6 95	6 09	9 80	0 0701	0 0012	0 0049	0 0042	0 0069	0 0277
Do	June 1	29 95	1 53	6 29	7 09	9 86	1662	0025	0105	0118	0164	0303
Do	June 15	29 50	1 35	5.70	10.35	10 82	4547	0061	0259	0470	0492	0432
Do	June 29	29 54	1 23	5 70	10 64	10 97	8980	0110	0512	.0956	0985	0446
Do	July 14	27 99	1 15	4 83	14 57	10 86	1 9294	0222	0932	2811	2095	0566
Do	Aug 1	22 09	1.08	4.09	16.02	10.11	3 6515	.0394	1493	.5850	3692	0454
Do	Aug 7	22 95	1 00	3 86	14 82	11 24	4 5567	0456	1759	.6754	5122	0441
Do	Aug 14	24 39	.96	3.29	11.05	13 26	5 6804	.0545	.1869	6277	.7532	0357
Do	Aug 21	25 91	95	3 43	9.51	13 91	7 1719	.0681	2460	.6821	9970	0333
Do	Aug 28	28 60	1 01	3 89	8 26	13 81	8 0795	0816	3143	6674	1 1158	0331
Shuck	Aug 14	26.51	1 29	4 87	12.03	12 86	2 5688	.0331	1251	3090	3303	0434
Do	Aug 21	23 99	1 47	5 47	12 67	12 23	3 1460	.0462	.1721	3986	3848	0400
Do	Aug 28	22 56	1.31	6 45	13 07	12 10	3 3759	0442	2177	4413	4085	0381
Shell plus kernel	Aug 14	23.15	.65	2 28	9.22	14 62	3 0188	0196	0688	2783	4413	0278
Do	Aug 21	28 47	.65	2 26	6.64	15 70	4 0632	0264	0918	2698	6379	0264
Do	Aug 28	36 60	72	2.00	5.32	14 08	4 7990	0346	0960	2074	6757	0249
Kernel	Aug 28	19.02	2 37	4 24	4 34	7 25	9013	.0214	0382	0392	0653	0162

While the data for 1933 show the same general trends as those for the few samples taken during the same period of fruit development in 1932, they also show fluctuations in concentrations of various constituents during the prefilling period that were not apparent in the previous data. For this reason the data for dry matter, ash, total sugars, and acid-hydrolyzable polysaccharides are presented graphically in figures 1 to 4. The data for nitrogen are not shown graphically because the amount of this constituent increased steadily throughout the prefilling period, as it did in 1932.

The percentage of dry matter in the whole fruit dropped rather suddenly in July, reaching a minimum on August 1. During this same period there was a rapid increase in the actual amount of dry matter per fruit, caused by the rapid increase in size. The percentage of dry matter in the whole fruit increased from August 1 to the end of the experiment, owing to the development of the shell and shuck. The data indicate that during August the shell development was somewhat greater than that of the shuck, since the dry matter in the shell plus kernel increased more rapidly than that in the shucks. The hardening of the shell occurred during the first 3 weeks of August. The kernel contained relatively little dry matter at the end of August, and not all of this was developed during the month. From the middle to the last of August the percentage of dry matter in the shuck decreased rapidly while the total amount per shuck increased. During this same period both the percentage and the amount of dry matter in the whole fruit and of the shell-plus-kernel samples increased. Thus, the whole fruit had its highest concentration of moisture on August 1, while the moisture concentration in the shuck increased rapidly during the latter part of August. In 1932 the percentage of moisture in the shucks increased through September, after which it decreased to the date of harvest.

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[4] THOR, CLIFFORD J. B., and SMITH, CHARLES L. See footnote 3.

A comparison of the changes in dry matter with rainfall records does not show consistent correlations. There was a sharp drop in the percentage of dry matter in the whole fruit from July 14 to August 1, 1933, during which time the rainfall was 6.98 inches. However, there was also an appreciable decrease in the percentage of dry matter in the whole fruit from June 29 to July 14, yet no appreciable amount of rain fell from June 1 to the latter date. The rainfall from August 1 to August 28 was only 0.32 inch, yet the percentage of dry matter in the shucks decreased from 26.51 percent to 22.56 percent from August 14 to August 28.

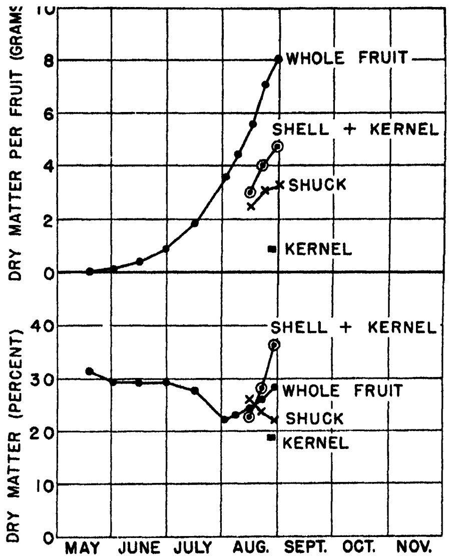

FIGURE 1.—Changes in dry-matter content of the pecan nut during the prefilling period.

The percentage of ash in whole fruits decreased regularly and rather rapidly to August 14, after which it started to increase, but the amount of ash per fruit showed a regular increase throughout the experiment. In the shuck the ash increased rapidly during the latter part of August both in amount and in percentage of the dry weight. In the shell-plus-kernel samples there was a small increase in amount of ash but a decrease in its percentage on the dry-weight basis. The increase in the percentage of ash in the whole fruit from August 14 to 28 was due largely to the rapid increase of ash in the shuck (fig. 2).

The changes in total sugar during the prefilling period are shown in figure 3. The percentage of sugars on the dry-weight basis increased rapidly in the whole fruit until August 1 and then decreased rapidly to the end of the experiment, but the actual amount of sugar increased regularly to August 7, after which it remained practically constant. The sugar in the shuck increased rapidly from August 14 to 28, while that in the shell-plus-kernel samples was decreasing. The drop in percentage of sugar in whole fruits was due to the rapid accumulation of dry matter of the shell and shuck. Since the amount of sugar in the whole fruit changed but slightly in August, an amount of sugar equivalent to the increased carbohydrate material of the shell and shuck must have been translocated from the tree to the fruit during the 3 weeks when the shell was hardening. The decrease in the sugar content in the shell-plus-kernel samples in late August may have been the result of its transformation to acid-hydrolyzable polysaccharides, which increased materially at this time.

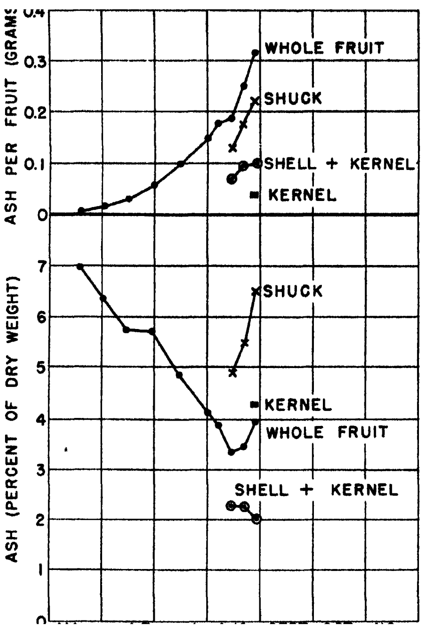

FIGURE 2.—Changes in ash content of the pecan nut during the prefilling period.

The ratio of total sugar to moisture in the whole fruit rose rapidly from May to the middle of July and then dropped equally rapidly, the maximum occurring just a little before the first visible signs of shell hardening. The occurrence of this sharp maximum in sugar concentration is probably related to the process of shell development rather than to the beginning of division of the fertilized egg as was suggested in the previous paper.

The concentration of acid-hydrolyzable polysaccharides on a dry-weight basis increased slightly during the rapid period of growth of the fruit, then dropped a little, and with the beginning of shell hardening early in August rose sharply again to concentrations considerably above previous levels. This is what would be expected, since pecan shells have been found to contain a relatively large amount of acid-hydrolyzable polysaccharides yielding chiefly *d*-xylose on hydrolysis.[5] The drop in concentration the latter part of July was due to the increased water content of the shuck during that time. On a weight-per-fruit basis the acid-hydrolyzable polysaccharides in the whole fruit simply increased in a regular manner throughout the entire prefilling period, as was indicated by the 1932 data. The data show that this increase was due largely to the increase of these substances in the shell and to a lesser extent to their increase in the shucks. The kernels contained very little acid-hydrolyzable polysaccharides on August 28.

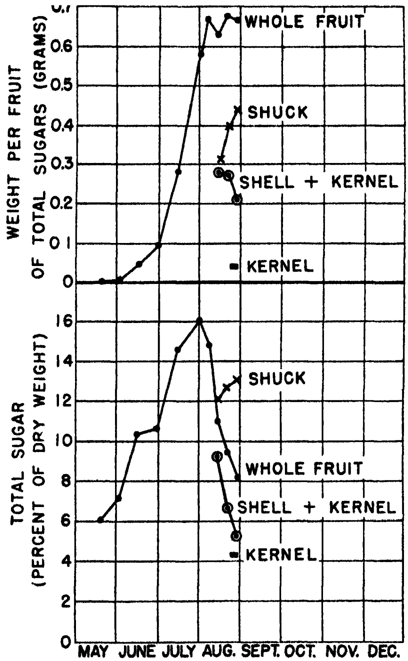

FIGURE 3.—Changes in total sugar content of the pecan nut during the prefilling period.

SUMMARY

The data presented for 1933 confirm the results for changes in composition of pecan fruits during the prefilling period of 1932. It is shown that the development of the shell is completed during this period, since a comparison of these data with those for later dates in 1932 shows relatively little change in the amounts of constituents after the filling process begins, except a loss of total sugars. The shell developed rapidly during August, showing complete hardening during the first 3 weeks of that month. This development was accompanied by a rapid increase in acid-hydrolyzable polysaccharides and dry matter and a decrease in total sugars. The nitrogen simply increased steadily with the development of the nuts.

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[5] THOR, CLIFFORD J. B., and SMITH, C. L. PECAN SHELLS AS A SOURCE OF D-XYLOSE. Jour. Amer. Chem. Soc. 56: 1640. 1934.

During the latter part of August there were rapid increases in total sugars and ash in the shuck, with lesser increases in dry matter and acid-hydrolyzable polysaccharides. By comparing the data for the 1933 season with those for later dates in 1932, it is shown that the shuck development was completed during the prefilling period, except for an increase in the ash content and slight changes in the total sugars and acid-hydrolyzable polysaccharides. The percentage of moisture in the shuck increased for some time after nut filling began in 1932, and was increasing at the beginning of this process in 1933.

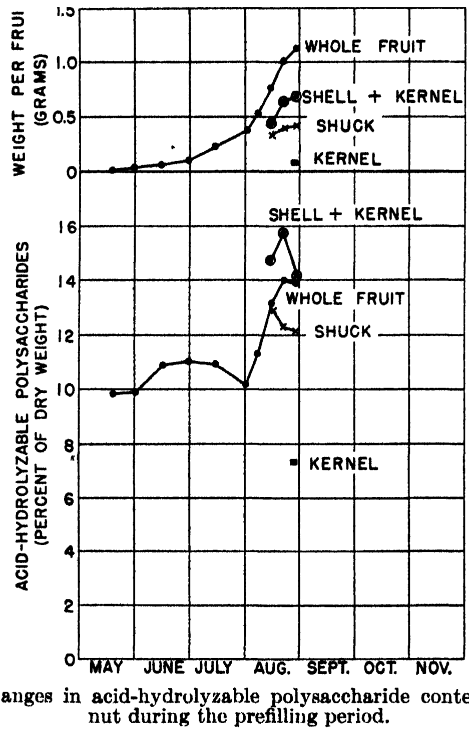

FIGURE 4.– Changes in acid-hydrolyzable polysaccharide content of the pecan nut during the prefilling period.

While the present data from frequent sampling show the same general trends as data from the few samples for the corresponding period in 1932, they also show the fluctuations in amounts of the constituents in the nut during the prefilling period.

DWARFING OF CUCURBITS SPRAYED WITH BORDEAUX MIXTURE[1]

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INTRODUCTION

Bordeaux mixture has long been recommended as a protective spray for cucurbits to discourage the cucumber beetle and diseases. Many farmers have been loath to use the material, however, reporting that the treatment is more deleterious to the plants than the pests are. Copper injury was long suspected. This suspected injury was combated by reducing the copper or increasing the lime content of the mixture. Still farmers have persisted in using other materials or no fungicides at all on their cucurbits.

Bordeaux injury on plants has been under investigation at this station for several years. The two most important elements in bordeaux injury are the lime (*6*)[3] and the copper (*9*). Papers on the effect of bordeaux mixture on transpiration (*3*) and bordeaux injury on tomatoes (*4*) have already been published. Wilson (*10*) has investigated bordeaux injury to cucurbits.

There are six distinct symptoms of bordeaux injury on cucurbits: (1) Dwarfing of the plants; (2) distortion of leaf margins, (3) yellowing of leaf margins followed by necrosis; (4) scorching of leaf lamina; (5) accelerated transpiration; and (6) defloration. This paper deals with the dwarfing and leaf-deformation aspects of the problem.

Since bordeaux mixture is prepared from three components, copper sulphate, lime, and water, the effect of these three substances was investigated first. Their effect was not clearly evident, however, until the effect of spray load and hydrogen-ion concentration was understood.

MATERIALS AND METHODS

The first problem that presented itself was to measure accurately the deleterious effects observed in the field. Field trials were ruled out for exploratory work because striped cucumber beetles proved so serious on the plants on which no insecticides were used that they vitiated conclusions. It seemed best to do as much of the work as possible in the greenhouse away from the disturbing influence of the beetles. The greenhouse work was conducted during the winter seasons of 1936 to 1938 inclusive, and some of the conclusions were checked during the summer seasons of the same years.

Chicago Pickling cucumbers or Honey Rock musk melons were planted in rows 6 inches apart and 4 feet long in greenhouse benches filled with electrically pasteurized soil (*2*) to keep damping-off at a minimum. Between crops the soil was treated with formalin (*1*). Seeds were sown about 1 inch apart at a strictly uniform depth so that a uniform emergence of seedlings was obtained. Sprays were applied with a 1-quart hand atomizer. Treatments were applied on single rows of about 40 plants replicated from four to six times. Drift was prevented by baffle boards on either side of each row. Sprays were applied approximately weekly; i. e., when seedlings emerged, when

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[1] Received for publication October 26, 1938, Journal Paper No. 292, New York State (Geneva) Agricultural Experiment Station.

[2] The writers are grateful to R. O. Magie and George Pearce for assistance. This research project was conducted in cooperation with the Crop Protection Institute.

[3] Italic numbers in parentheses refer to Literature Cited, p. 926.

FIGURE 1.—Dwarfing of cucumber plants by various sprays (series 4a, table 1): *a*, Copper silicate; *b*, red cuprous oxide; *c*, copper silicate plus Bancroft clay; *d*, red cuprous oxide plus Bancroft clay; *e*, Bancroft clay alone; *f*, nonsprayed.

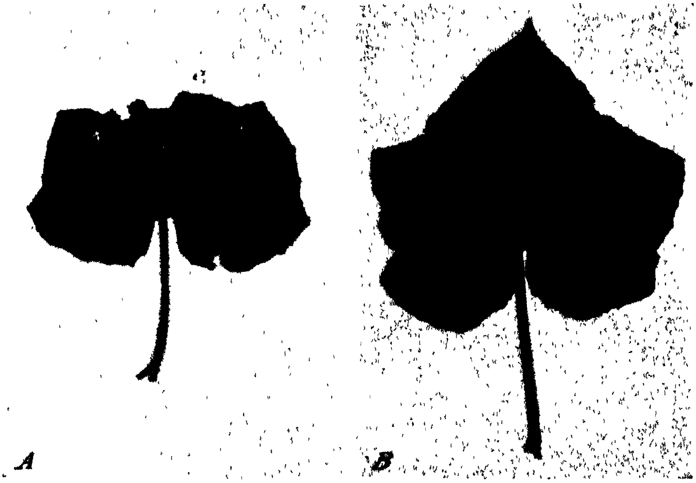

FIGURE 2.—Leaf deformation of cucumber caused by bordeaux mixture: *A*, Sprayed leaf; *B*, nonsprayed leaf.

the first true leaf appeared, and when the second true leaf appeared. A photograph of a typical test appears in figure 1.

The basic spray was 4–4–50 or 5–5–50 [4] bordeaux prepared with hydrated lime. The word "lime" in the text refers to commercial or laboratory-made hydrated lime in all cases. All sprays were made up in tap water. The other materials were applied in equivalent copper concentrations or in equivalent spray loads. Plants used as checks were sprayed with tap water.

Plants were thinned at intervals by clipping them at the ground line and weighing them while green. From one to three harvests were made from each experiment. The separate harvests are referred to as "series" in the tables and text. By conducting the cultural operations carefully and by using pasteurized soil to obviate variations due to damping-off of seedlings, this technique proved adaptable, simple, and reproducible for measuring dwarfing and leaf deformation.

RESULTS

Dwarfing of cucumber plants by various sprays is illustrated in figure 1. The leaf-deformation symptom is shown in figure 2 in comparison with a normal leaf. Both dwarfing and leaf deformation are closely allied. They seem to be produced by the same factors. Data obtained in a greenhouse study of some of the factors that influence dwarfing of cucurbits are given in table 1.

EFFECT OF COPPER

It is very difficult to obtain an adequate evaluation of the copper factor in bordeaux injury. The use of copper sulphate alone is hardly practicable except as an indication, because the copper is so soluble as to be very toxic, producing necrosis and stunting which are far in excess of that produced by bordeaux and which are not typical of bordeaux injury.

The effect of the copper factor has been studied (1) by using insoluble copper compounds with and without lime, and (2) by varying the copper content of bordeaux and keeping the lime constant.

TABLE 1.—*Effect of various sprays [1] on growth of cucurbits [2] in the greenhouse*

[Data expressed as average green weight per plant in grams]

Materials used in spray	Weight per plant for date of harvest and series No. indicated								
	Mar. 2, 1936, No. 1	Mar. 9, 1936, No. 1a	Mar 13, 1936, No 1b	Mar 9, 1936, No. 2	Mar. 14, 1936, No 2a	Apr. 8, 1936, No. 3	Apr. 15, 1936, No. 3a	Apr. 22, 1936, No. 3b	May 4, 1936, No. 4
Water	0.684	1.61	2.60	1.48	2.65	1.33	2.65	5.76	0.87
Bordeaux	.488	1.49	2.02	1.19	1.74	1.15	2.05	4.15	.75
Red cuprous oxide	.605	1.73	2.67	1.49	2.39	1.33	2.46	5.29	[3] .85
Red+Bancroft clay	------	------	------	------	------	------	------	------	.63
Copper silicate (Coposil)	.520	1.29	1.75	1.27	1.90	1.31	2.34	4.74	.71
Coposil + Bancroft clay	------	------	------	------	------	------	------	------	.61
Lime [6]	.582	1.51	2.06	1.51	2.27	1.36	2.67	5.39	------
Lime [7]	------	------	------	------	------	1.25	2.15	4.52	------
Bancroft clay	------	------	------	------	------	------	------	------	.81

See footnotes at end of table.

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[4] Spray formulas are employed to simplify the text. A formula 4–4–50 bordeaux means 4 pounds of copper sulphate pentahydrate, 4 pounds of hydrated lime, and 50 gallons of water, and 1–50 of any material means 1 pound of the material in 50 gallons of water.

TABLE 1.—*Effect of various sprays on growth of cucurbits in the greenhouse*—Continued

[Data expressed as average green weight per plant in grams]

Materials used in spray	Weight per plant for date of harvest and series No. indicated							
	May 12, 1936, No. 4a	May 28, 1936, No. 5	Apr. 15, 1937, No. 6	Apr. 24, 1937, No. 6a	Apr. 15, 1937, No. 7	Apr. 24, 1937, No. 7a	May 13, 1937, No. 8	June 5, 1937, No. 9
Water	4.13	1.73	1.94	7.32	1.82	6.18	4.85	13.65
Bordeaux	3.25	------	1.69	5.47	1.51	4.11	3.45	11.24
Red cuprous oxide	[3] 3.98	1.65	1.89	6.87	------	------	4.37	13.09
Red+calcium arsenate	------	------	------	------	------	------	3.91	12.93
Red+lead arsenate	------	------	------	------	------	------	------	10.37
Red+lime	------	1.52	------	------	------	------	4.06	13.07
Red+lime+Bancroft clay	------	------	------	------	------	------	4.19	11.86
Red+Bancroft clay	1.93	1.32	------	------	1.54	4.20	3.68	11.34
Red+sulphur	------	1.71	------	------	------	------	------	------
Red+diatomaceous earth	------	1.69	------	------	------	------	------	------
Red+flour	------	1.69	------	------	1.83	6.14	------	12.21
Red+talc	------	------	------	------	1.64	5.93	4.24	------
Red+Wyogel	------	------	------	------	------	------	4.17	------
Red+gypsum	------	------	------	------	------	------	4.41	13.30
Red+Celite	------	------	------	------	------	------	4.44	------
Red+Hammil & Gilispie clay	------	------	------	------	------	------	4.01	12.60
Red+magnesium oxide [4]	------	------	------	------	------	------	4.08	12.38
Copper oxychloride [5]	------	------	1.91	6.59	------	------	------	------
Copper+talc	------	------	------	------	1.90	5.44	------	------
Copper+clay	------	------	------	------	1.62	4.68	------	------
Copper+flour	------	------	------	------	1.79	5.89	------	------
Copper silicate (Coposil)	2.85	------	1.84	6.34	------	------	------	------
Coposil+Bancroft clay	1.77	------	------	------	------	------	------	------
Coposil+bentonite	------	------	1.82	6.30	------	------	------	------
Coposil for citrus	------	------	1.91	5.99	------	------	------	------
Lime [6]	------	------	------	------	------	------	3.65	12.34
Lime [7]	------	------	------	------	------	------	3.24	11.12
Bancroft clay	3.46	------	1.78	6.01	------	------	------	------
Talc	------	------	1.86	6.44	------	------	------	------
Wheat flour	------	------	1.88	6.37	------	------	------	------
Derris	------	------	1.73	6.16	------	------	------	------
Calcium arsenate	------	------	1.70	5.92	------	------	3.98	12.69
Lead arsenate	------	------	------	------	------	------	------	11.39

[1] Sprays were made up at the rate of 1.25 pounds of copper (as metallic) in 50 gallons of water except series Nos. 6 to 9, where the rate was 1 pound. Other materials, such as lime, etc , were added at the rate of 5 pounds in 50 gallons, except series Nos. 6 to 9, where 4 pounds was used.

[2] All data were obtained with cucumbers, except series Nos. 3, 3a, and 3b, in which muskmelons were used.

[3] Red cuprous oxide, 5 to 50 gallons.

[4] Magnesium oxide, only 4 ounces to 50 gallons.

[5] Grasselli A copper oxychloride.

[6] Lime equivalent to lime content of bordeaux.

[7] Lime equivalent to spray load of bordeaux.

The so-called insoluble copper compounds like red cuprous oxide (electrolytic grade, Cuprocide) or copper oxychloride (Grasselli compound A) did not seriously stunt cucumber foliage in the greenhouse (table 1), nor did they cause leaf deformation. Copper silicate (Coposil), however, was found to stunt plants considerably in almost every one of the 12 series in which it was included (table 1). Plants sprayed with copper silicate were invariably smaller than those sprayed with red cuprous oxide or copper oxychloride. The reasons for this are not clear, but the effect may have been owing as discussed below, to some diluent in the copper silicate and not to the copper.

Three experiments were made in which the effect of copper in bordeaux was measured by varying the copper content and keeping the lime constant (table 2). In the first two tests commercial hydrated lime was used. The degree of carbonation of this lime was not determined although one sample obtained from stock the same winter showed 38 percent of carbonate. Lime for the third test was burned and slaked. Observation of the curves (fig. 3) for the three experiments indicates two possible conclusions (1) that copper has little or no effect, or (2) that this type of test does not demonstrate it very clearly. The bimodal shape of the curves in two of the tests indicates that factors other than the copper were chiefly responsible for their shape. As the copper content of the spray increased, the spray load increased and the pH value decreased. The effect of these factors on the shape of the curve is discussed below.

TABLE 2.—*Effect of hydrogen-ion concentration and spray load of various mixtures on growth of cucumbers*

Material	Spray formula[1]	Spray load, quantity to 50 gallons	pH	Green weight per plant		
				May 1937[2]	June 1937[3]	February 1938
		Pounds		*Grams*	*Grams*	*Grams*
Water		0	7.5	4.85	13.65	1.660
Bordeaux	4-1-50	5.0	4.4	3.72	11.10	1.378
	4-2-50	6.0	11.7	3.94	11.93	1.460
	4-4-50	8.0	12.3	3.45	11.24	1.317
	4-6-50	10.0	12.3	3.44	11.19	1.159
	4-8-50	12.0	12.3	3.43	10.73	1.088
	1-4-50	5.0	12.3	4.04	12.08	1.247
	2-4-50	6.0	12.3	3.73	11.22	1.276
	6-4-50	10.0	12.1	3.36	11.11	1.408
	8-4-50	12.0	11.7	3.75	11.22	1.471
Cu_2O	1.2-50	1.2	6.40	4.37	13.09	
Cu_2O+Bancroft clay	1.2-5-50	6.2	3.56	3.68	11.34	
Cu_2O+Hammil & Gilispie clay	1.2-5-50	6.2	4.65	4.01	12.60	
Cu_2O+Celite	1.2-5-50	6.2	7.55	4.44		
Cu_2O+gypsum	1.2-5-50	6.2	8.66	4.44	13.30	
Cu_2O+talc	1.2-5-50	6.2	8.88	4.24	12.69	
Cu_2O+magnesium oxide	1.2-.25-50	2.0	9.70	4.08	12.38	
Cu_2O+calcium arsenate	1.2-5-50	6.2	9.70	3.91	12.93	
Cu_2O+Wyogel	1.2-5-50	6.2	10.49	4.17		
Cu_2O+lime	1.2-5-50	6.2	12.30	4.06	13.07	
Cu_2O+lime+clay	1.2-2.5-2.5-50	6.2	12.30	4.19	11.86	
Lime	4-50	4.0	12.30	3.65	12.34	
	8-50	8.0	12.30	3.29	11.12	

[1] The numbers in the formula refer to the quantity of the ingredients in pounds per 50 gallons; the first figure shows copper, the second, lime for bordeaux or diluent for Cu_2O.

[2] This is series 8, table 1

[3] This is series 9, table 1.

These data indicate that insolubilized copper is not a primary cause of dwarfing and leaf deformation on cucumber plants. The copper in bordeaux is insolubilized with lime in excess. It was important, then, to investigate the effect of the excess lime.

EFFECT OF LIME

When the lime content of a spray, in which only lime was used, was made equivalent to the lime content of bordeaux mixture, the plants were dwarfed and the leaves deformed, but the effect was not so great as that of bordeaux (table 1). If the content was increased so as to equal the spray load of the bordeaux the stunting increased, but still was not quite so serious as when bordeaux was used. Leaf deformation also increased with an increase in lime.

The effect of the lime was studied also by varying its concentration in bordeaux and keeping the copper content constant (table 2 and fig. 4). The curves from data in three tests (fig. 4) all show that, generally speaking, growth fell off as lime content of the bordeaux increased.

The only exception was that growth did not fall off as the lime was increased from 4–1–50 to 4–2–50. This increase in growth with increase in lime was not caused by a direct beneficial effect of the lime on the growth, however, but rather by a reduction in soluble copper, i. e., a reduction in copper injury. The soluble copper in 4–1–50 bordeaux scorched the foliage so badly as to stunt the plants.

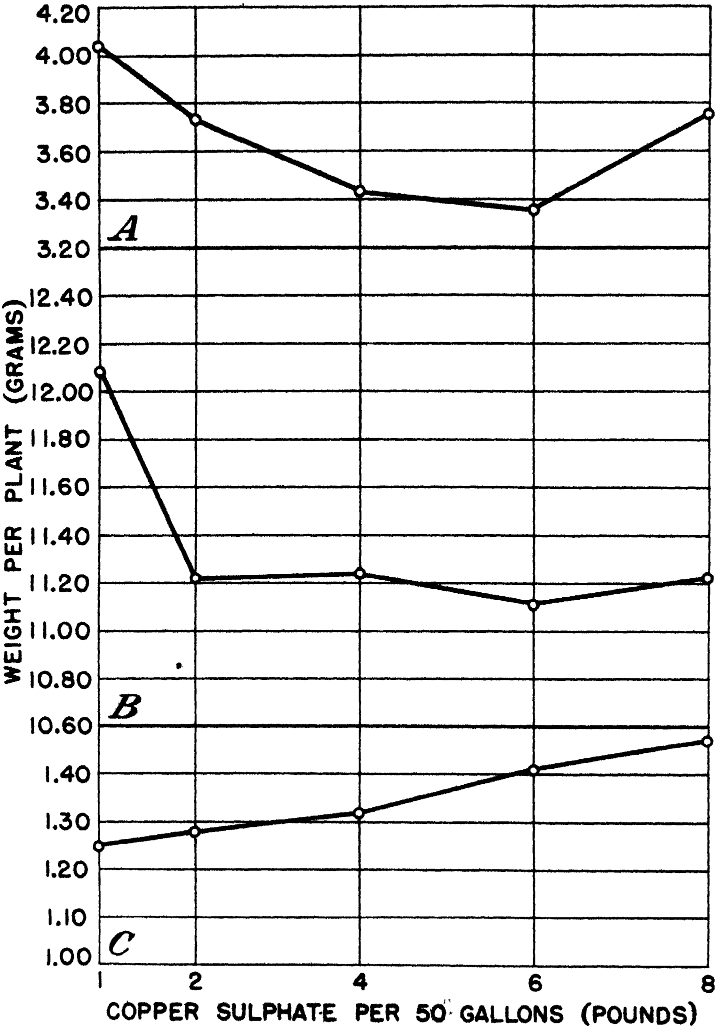

FIGURE 3.—Effect of varying copper content of bordeaux used in spray on growth of cucumber plants (table 2). (Four pounds of lime in each 50 gallons: *A*, data from May 1937 experiment; *B*, data from June 1937 experiment; *C*, data from February 1938 experiment.

The fact that growth diminished as the lime content of the bordeaux increased above 4–2–50 was not proof that the lime was wholly responsible, however, because both spray load and pH value also increased

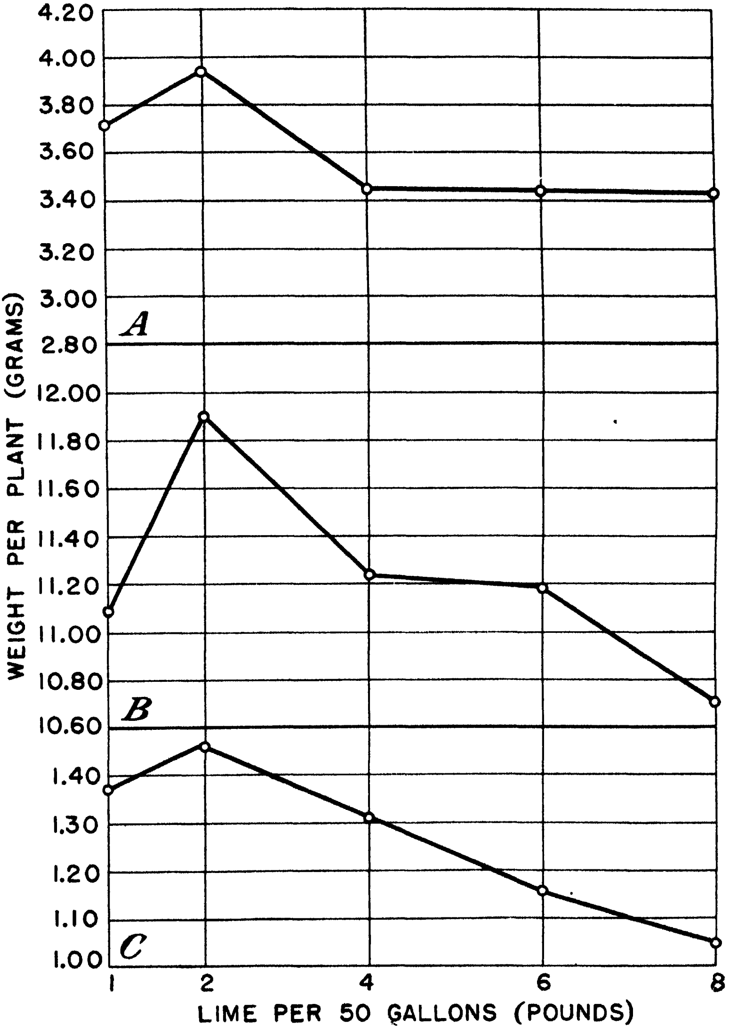

FIGURE 4.—Effect of varying lime content of bordeaux used in spray on growth of cucumber plants (table 2): *A*, Data from May 1937 experiment; *B*, data from June 1937 experiment; *C*, data from February 1938 experiment.

and both of these are important in the dwarfing caused by the bordeaux as shown later.

If soluble copper compounds do not stunt cucumber foliage seriously or deform the leaves, and if lime does, it can be assumed that the active agent is the calcium in the lime, or the alkalinity or both. The part played by hydrogen-ion concentration is discussed below.

The effects of excess calcium absorption into the tissues are not clearly understood. Nutritional studies with calcium usually deal with deficiencies. A low-lime soil was put into the greenhouse and fertilized well (at the rate of 2,000 pounds of 5–10–12 per acre). Lime was added to half of this soil at the rate of 3 tons per acre. Three consecutive crops of seedlings were grown in the usual way (table 3).

TABLE 3.—*Effect of liming soil on growth of cucumbers, 1938*

Series No.	Date harvested	Average weight per plant in—	
		Limed soil	Nonlimed soil
		Grams	*Grams*
1	Mar 18	3.20	5.64
2	Apr 8	3.50	4.16
3	May 3	7.17	8.18

The plants in the limed soil were drastically dwarfed, and the leaves were deformed in a fashion resembling bordeaux deformation. The dwarfing, as shown by the difference between the plants in the limed and nonlimed soils diminished with succeeding crops. This experiment shows that a high lime content in the soil like a high lime content in a spray will dwarf cucumbers and deform the leaves. It still leaves unanswered, however, the question as to whether the dwarfing is due to excess calcium absorption or to some indirect effect of high alkalinity.

The addition of red cuprous oxide seemed to lessen the dwarfing effect of a lime spray (series 8 and 9, table 1). This would be expected if dwarfing were caused by penetration of calcium ions into the tissues, because the addition of copper oxide to calcium oxide solution would depress the calcium-ion concentration and thus reduce the injury. This reduction in calcium-ion concentration would result from the action of the oxide anion common to both salts. It is possible also that there is a direct antagonism in the tissues between the calcium and copper ions.

Calcium arsenate, which carries calcium but no oxide, served to check the ionization hypothesis. Used alone it reduced growth somewhat (series 6, 6a, 8, and 9, table 1), but, as expected, the addition of red cuprous oxide to it did not reduce the injury appreciably, because copper oxide and calcium arsenate do not carry a common anion.

EFFECT OF WATER

It is difficult to obtain adequate experimental comparisons of materials with and without water, because it is difficult to apply comparable spray loads. Bordeaux and copper-lime dust were compared in the field in 1936 and 1937. Three seedling harvests from six replicate plots were made in 1936 and one harvest from five replicate plots in 1937. The paired average weights in grams per plant for those covered with 25–75 copper-lime dust and bordeaux, respectively, were as follows: 3.54–3.23, 9.88–7.80, and 24.7–24.2 in 1936; and 12.99–9.88 in 1937. These data show that water aggravates the dwarfing injury from copper sulphate-lime mixtures. Since the water acts as a vehicle, its effect is indirect.

EFFECT OF DILUENTS AND INSECTICIDES

The diluents were used alone and in combination with red cuprous oxide, copper oxychloride, and copper silicate. The materials tried were as follows: Calcium arsenate, lead arsenate, Bancroft clay, Hammil & Gillispie clay, bentonite, Wyogel (Wyoming bentonite), derris, diatomaceous earth (Celite), wheat flour, gypsum, lime, sulphur, and talc (tables 1 and 2).

Of the diluents, wheat flour, talc, diatomaceous earth, and gypsum gave least dwarfing. All of the clays including the bentonites stunted the plants somewhat and deformed the leaves. On this basis it was probably the bentonite diluent in the copper silicate that caused it to stunt the plants. The most injurious clay was Bancroft clay, a material sometimes used as a diluent for fungicides and insecticides (*3*). It is interesting that the addition of red cuprous oxide to a lime spray tended to reduce the injury caused by the lime, but the addition of red cuprous oxide to Bancroft clay spray increased both the stunting and the leafy deformation caused by the clay (table 1, series 4, 4a, 5, 8, 9, and fig. 1). A combination of copper silicate and Bancroft clay was more injurious also than either alone (table 1, series 4, 4a, and fig. 1).

Of the insecticides, derris was much the least injurious, followed by calcium and lead arsenate.

EFFECT OF SPRAY LOAD

A clear picture of the effect of sprays on plants cannot be obtained unless the spray load is held constant. Spray load has been defined (*3*) as the amount of material applied to the leaf. The amount actually retained was not determined in these tests. Since no detergents were added unless otherwise stated, it was assumed that the application and retention of all sprays was essentially proportional to the amount of material in 50 gallons of water.

The data on lime alone indicate that increasing the spray load increased dwarfing (table 1, series 3, 3a, 3b, 8, 9, and table 4).

The three experiments (table 2) varying the copper and lime contents of bordeaux were, among other things, experiments on variable spray load. When curves were plotted for the data (fig. 5) using mixtures of the same hydrogen-ion concentration it became clear that growth generally decreased as spray load increased whether the copper or the lime was varied.

Perhaps the curves in figure 5 would have been more conclusive had they not been plotted from data in which both copper and lime ratios shift. Accordingly a test was made in the greenhouse (table 4) where both spray load and copper-lime ratios were shifted independently. Copper sulphate-lime ratios of 2–1, 1–1, and 1–2 were compared, each with spray loads of 3, 6, and 12 pounds per 50 gallons of water. These data confirm those in figure 5 showing that growth fell off as spray load increased.

Another test was made in which the spray load was varied by using amendments designed to increase the run-off and thus reduce the spray load. In this test lime at 8–50 was sprayed alone, plus soybean flour, and plus Grasselli spreader-sticker (SS3). The relative run-off for the three mixtures was determined in the laboratory by using a cellulose nitrate film on glass and a laboratory sprayer similar to that described by Horsfall, Marsh, and Martin (*5*). The average time in seconds in three trials for the first drop of spray to run down was determined for the three mixtures. These data are compared with those for plant growth of the sprayed plants (table 5).

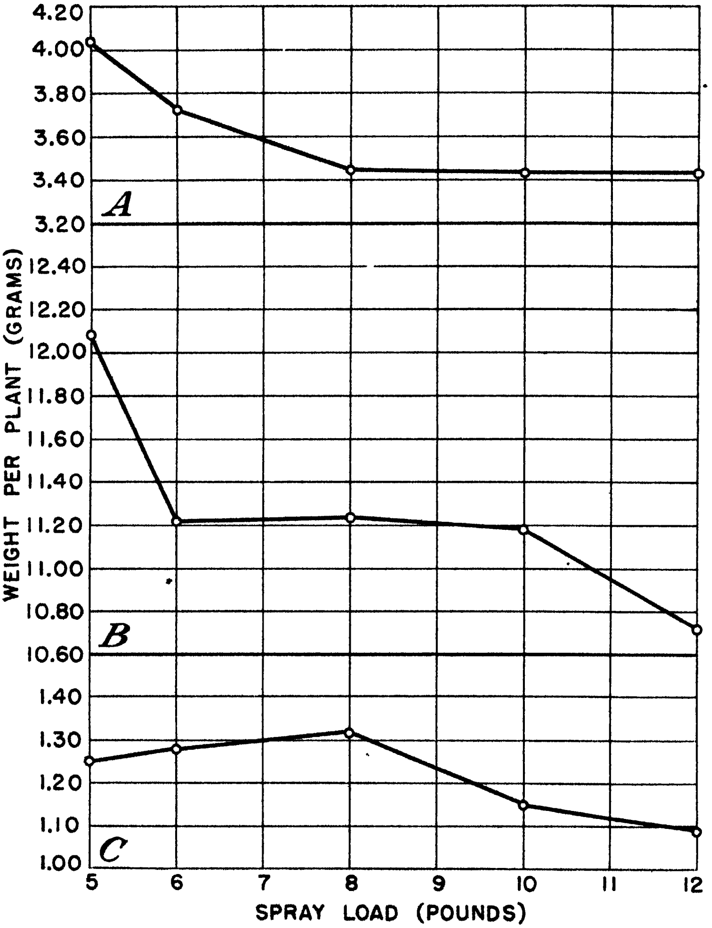

FIGURE 5.—Effect of varying spray load of bordeaux (pounds per 50 gallons) at same pH value on growth of cucumber plants (table 2). *A*, Data from May 1937 experiment; *B*, data from June 1937 experiment; *C*, data from February 1938 experiment.

TABLE 4.—*Effect of spray load and copper lime ratios in bordeaux mixtures on growth of cucumbers*

Copper sulphate-lime ratio	pH	Bordeaux formula [1]	Spray load, quantity per 50 gallons	Average green weight per plant
			Pounds	*Grams*
2 1	11.7	2-1-50	3	3.54
		4-2-50	6	3.40
		8-4-50	12	3.28
Average				3.41
1 1	12.3	1½-1½-50	3	3.49
		3-3-50	6	3.40
		6-6-50	12	2.59
Average				3.16
1 2	12.3	1-2-50	3	3.36
		2-4-50	6	3.03
		4-8-50	12	2.55
Average				2.98
	12.3	0-3-50	3	3.04
		0-6-50	6	3.02
		0-12-50	12	2.92
Average				2.98
Average per spray load			3	3.36
			6	3.21
			12	2.83

[1] Expressed as pounds of copper sulphate and lime per 50 gallons of water.

Both the detergents increased run-off and thus lessened the spray load, which in turn, lessened the dwarfing. This is further evidence that spray load is important in dwarfing.

EFFECT OF HYDROGEN-ION CONCENTRATION

It was interesting to study the effect of hydrogen-ion concentration on growth and leaf injury since lime, which is alkaline, and Bancroft clay, which is acid, both gave stunning and leaf deformation. The effect of hydrogen-ion concentration has been studied (1) by varying the hydrogen-ion concentration of bordeaux, (2) by varying the hydrogen-ion concentration of red cuprous oxide suspensions prepared with different diluents, and (3) by using diluents of different hydrogen-ion concentrations without copper (tables 1 to 4).

TABLE 5.—*Effect of amendments to a lime spray on growth of cucumbers and run-off (i. e., spray load)*

Material	Spray formula [1]	Average green weight per plant	Average time for run-off
		Grams	*Seconds*
Lime alone	8-50	7.08	34.0
Lime+soybean flour	8-1-50	7.58	12.7
Lime+SS3 [2]	8-0.19-50	7.68	16.7

[1] Expressed as pounds of materials per 50 gallons of water.
[2] Grasselli spreader-sticker.

The hydrogen-ion concentration of the various bordeaux mixtures of approximately equal spray load was plotted against growth of cucumber plants for three experiments (fig. 6). Since no neutral bordeaux was prepared, the growth of the water-sprayed plants was inserted in the curves to establish the approximate shape of the midpart of the curves. Both acid and alkaline bordeaux reduced growth.

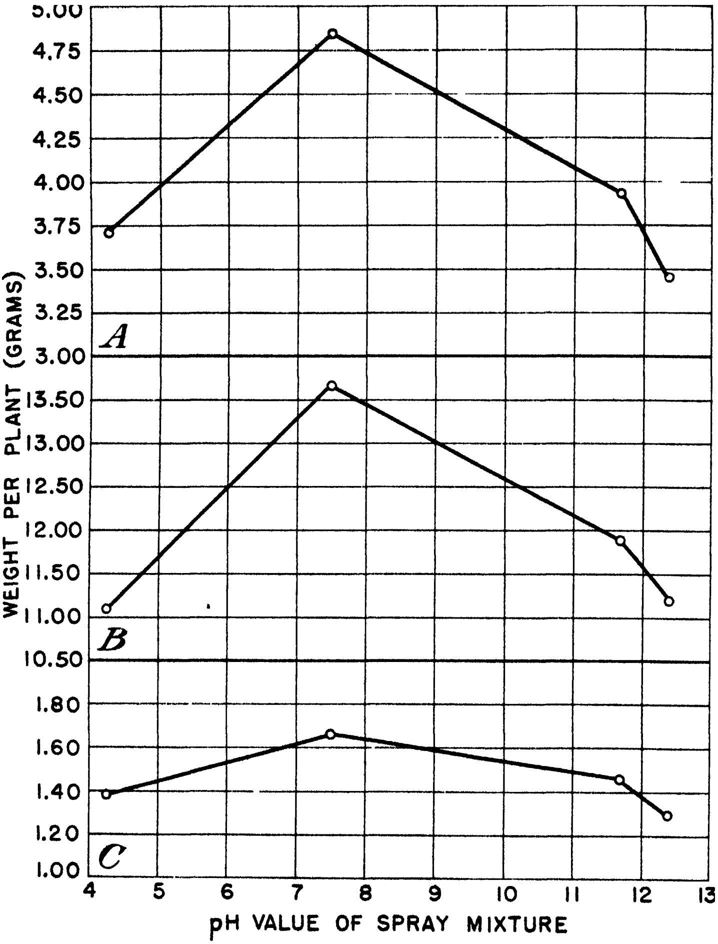

FIGURE 6.—Effect of varying the hydrogen-ion concentration of bordeaux mixture on growth of cucumber plants (table 2). The point for pH 7.5 is located by using the growth of water-sprayed checks: *A*, Data from May 1937 experiment; *B*, data from June 1937 experiment; *C*, data from February 1938 experiment.

The pH value of bordeaux rises sharply from 4.4 to 11.7 as the formula shifts from 4–1–50 to 4–2–50. In other words a single pound of lime increases the pH value by more than 7 units. Since bordeaux mixture is an unbuffered system, only a small quantity of lime is required to lift the pH value of the mixture practically to that of limewater once the copper has been insolubilized.

This peculiarity of bordeaux left a wide gap in the growth data between pH 4.4 and 11.7—a gap only partially filled by the use of data from water-sprayed plants. The fact that growth fell so rapidly when the pH value increased from 11.7 to 12.3 indicated that the peak of the curve was somewhere below pH 11.7. This was indicated also by the growth of the water-sprayed checks.

A series of sprays over the pH range were prepared using red cuprous oxide as an "insoluble copper" prepared with various diluents (table 2). The spray load was kept constant at 1.2–5–50. The copper concentration was held constant at 1 pound of metallic copper per 50 gallons.

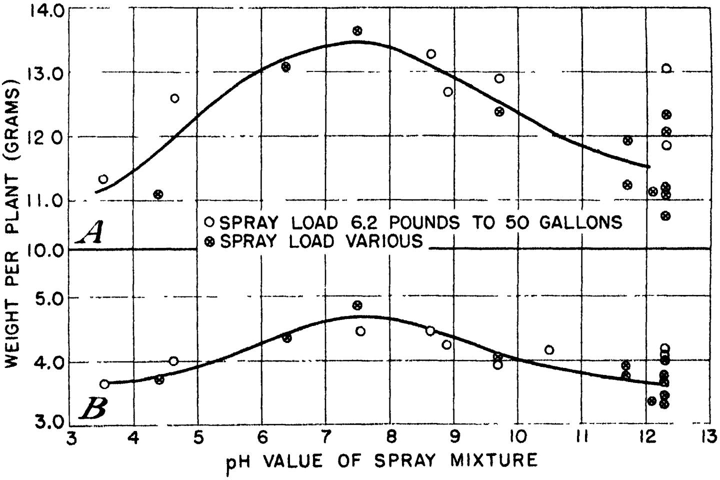

FIGURE 7.—Effect of hydrogen-ion concentration of various spray materials on growth of cucumbers (table 2): *A*, Data from June 1937 experiment; *B*, data from May 1937 experiment.

Curves (fig. 7) were drawn by inspection from three different experiments. The points for red copper oxide with diluents are shown as small circles. For the sake of completeness data from all other materials including bordeaux in the same tests are inserted on the curves as small crosses. The curves are essentially the same as those obtained with bordeaux alone showing poor growth at both high and low pH values. In these curves, however, the gothic shape is rather clearly defined. The optimum growth of cucumber plants was obtained with a spray mixture approximating pH 7.

Other data on the effect of miscellaneous materials (table 1) sprayed by themselves onto cucumber plants are interesting in this connection. As was pointed out above, lime alone, which is alkaline, and Bancroft clay alone, which is acid, both dwarfed cucumbers. Three distinct materials in the same experiment (table 1, series 6 and 6a), acid Bancroft clay, neutral red cuprous oxide, and slightly alkaline talc, gave the typical curve where growth was smaller with either the acid or alkaline material than with the neutral material. Critical observation of the data shows that the differences were small, but nevertheless the trend was the same in both series as it was in the two larger experiments (table 2) conducted 1 year later. Whatever be the statistical significance of an individual point on a curve, trends are significant especially if they appear in different experiments conducted under different conditions.

It appears to follow then, that growth of cucumbers is influenced by the hydrogen-ion concentration of the spray mixture irrespective of whether copper is present or not. A pH value below neutral probably exaggerates copper injury by aiding copper solubilization. The addition of acid Bancroft clay, for example (series 4, 4a, 5, 8, 9, table 1 and figure 1), to red cuprous oxide or copper silicate stunted cucumber plants and deformed the leaves more than when any of the three materials were used alone. Conversely, a pH value above neutral probably reduces copper injury because it reduces copper solubilization.

The striking relation of hydrogen-ion concentration of the spray mixture to dwarfing explains several observations. The dwarfing effect of the copper silicate was probably an effect of hydrogen-ion concentration because the sample showed a pH value at spray dilution of 8.38. From the curves it is plain that copper silicate at pH 8.38 should cause more dwarfing than red cuprous oxide at pH 6.40. The dwarfing from the various clays used was probably owing to the fact that none of them was near neutral in hydrogen-ion concentration. Some were above neutral, and others were below.

The derris, flour, talc, and gypsum diluents were relatively harmless to the plants because at spray dilution the pH value was practically 7.

DISCUSSION

There is a striking similarity between the factors for dwarfing caused by bordeaux and for the effects of bordeaux on transpiration. Neither factor was very much affected by the insolubilized copper materials. Both were favored by the lime and the water in bordeaux, and both were affected similarly by the acidity or alkalinity of the spray.

The similarities are concerned fundamentally with the effects of the hydrogen-ion concentration of the sprays. It has been suggested that the effect of bordeaux on transpiration (*3*) results from the dissolution of the plant cuticle by acids and alkalis. Increased transpiration is known to dwarf plants. It might be concluded that dwarfing in this case is a simple result of the effect of bordeaux on transpiration. If so, the search for the cause of the dwarfing induced by bordeaux need go no further.

Several facts disturb the simplicity of this conclusion: (1) The seedlings were growing in moist soil in a greenhouse under optimum conditions. Perhaps they could keep themselves supplied with enough water for growth even in the face of a rapid outgo. (2) Red cuprous oxide reduced the dwarfing, but not the transpiration effects of a lime spray. (3) Increasing spray load reduced stomatal transpiration and increased cuticular transpiration, but it uniformly decreased growth.

If dwarfing has other causes than accelerated transpiration, it must be due to an internal absorption of an overdose of copper or calcium from the bordeaux. Horsfall, Magie, and Suit (*4*) report that copper stiffens the tissues of tomatoes. This effect may have caused the small amount of dwarfing occasioned by the insolubilized copper materials. The part played by the copper component of bordeaux was probably small because insolubilized copper compounds did not cause much dwarfing and because copper-free materials did cause it. The lime element in bordeaux cannot be eliminated so readily.

Evidence is presented here and elsewhere (*4*) that calcium penetrates the tissues—hardens, and dwarfs them. All of the materials that showed much dwarfing contained some calcium although it may have been tightly bound up in some of the clays.

Assuming that excess calcium has a dwarfing effect, it is interesting to consider how calcium penetrates the tissue and how it dwarfs growth. The possible avenues of entry are the stomata and the cuticle. If the cuticle is concerned, only those calcium-containing materials with an acid or an alkaline reaction should cause dwarfing, because they are presumably the only ones that would injure the cuticle sufficiently for penetration. Such was the case. Dwarfing was negligible with gypsum and talc, both of which gave an essentially neutral spray. These materials also should have caused dwarfing if the stomata had been the avenue of entry.

Calcium probably reduces growth by curtailing the cell expansion phase. Sayre and Nebel (*8*) have shown that calcium taken in by a pea plant from water culture hardened the tissues of the ovule, presumably by combining with constituents of the middle lamella. It also thickened the cell walls.

The evidence from research on transpiration (*3*) and photosynthesis (*7*) indicates that sprays clog stomata. Therefore, photosynthesis is decreased, and growth is curtailed.

The three basic causes of the dwarfing induced by bordeaux can now be traced outward from known facts as follows: (1) Excessive transpiration of plants is known to cause dwarfing. Therefore, the dwarfing may result from the effect of bordeaux on transpiration, which appears to be a case of water loss through an injured cuticle (*3*). (2) On the other hand, calcium in the lime could pass into the plant through the same injured cuticle. Calcium is known to harden tissues. This could prevent cell enlargement and result in dwarfing. Therefore, the dwarfing in this case may partly accompany and partly be a result of the effect of bordeaux on transpiration. (3) Bordeaux may clog the stomata, curtail photosynthesis, and thus reduce growth.

PRACTICAL ASPECTS

From the standpoint of the practical grower the data presented herein indicate that both acid and alkaline sprays or dusts should be avoided on cucumbers and melons since these plants are dwarfed by them. A neutral bordeaux is an impractical recommendation in the hands of most growers because the margin of safety is too narrow. A difference of an ounce or two of lime would cause serious copper burn. A small amount of carbonation would give the same result. One of the insolubilized copper compounds would be preferable, although some of these are less satisfactory than others. Calcium arsenate appears to be the best insecticide consistent with expense, although some attention might be paid to making this material so that it has a neutral reaction in water suspension.

Diluents are also important. So-called inert carriers frequently are far from inert when applied to cucurbits. Many of them dwarf the plants. The clays as a group and lime seem to be out of the question from that standpoint. The safest carriers that were tried were diatomaceous earth, gypsum, talc, and wheat flour. Gypsum and diatomaceous earth did not run through the duster as well as talc or wheat flour, but flour is expensive. That leaves only talc.

The precise formula for making dusts or sprays of these materials must await extensive field research.

SUMMARY

There are six symptom types of bordeaux injury on cucurbits: Dwarfing, leaf deformation, yellowing of leaf margins followed by necrosis, scorching of leaf lamina, accelerated transpiration, and defloration.

The copper factor in a bordeaux mixture does not seem to be involved in bordeaux dwarfing unless the copper is solubilized by low hydrogen-ion concentration.

The lime factor definitely dwarfed cucurbits and deformed the leaves.

When water was added to a copper sulphate-lime mixture, dwarfing and leaf deformation were enhanced.

Increasing spray load increased injury.

The most significant factor in dwarfing and leaf deformation appears to be the hydrogen-ion concentration. If the hydrogen-ion concentration of the spray mixture was shifted very far either way from neutral, dwarfing was increased whether copper was present in the spray mixture or not.

The calcium ion also may possibly cause dwarfing by hardening young cells and preventing their enlargement.

The basic cause of dwarfing is discussed. From data available it is suggested that the dwarfing may be due to (1) physiological drought in the tissues induced by the effect of bordeaux transpiration, (2) to action of calcium ions in hardening the tissues, or (3) to reduced photosynthesis, or to a combination of these causes.

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INDIVIDUAL AND BREED VARIATIONS IN PIGS ON RATIONS DEVOID OF VITAMIN D[1]

By D. W. JOHNSON, *assistant animal husbandman, Division of Animal and Poultry Husbandry,* and L. S. PALMER, *agricultural biochemist, Division of Agricultural Biochemistry, Minnesota Agricultural Experiment Station*

INTRODUCTION

That a deficiency of vitamin D often occurs in swine during the winter under farm conditions is suggested from numerous reports received from farmers in Minnesota of so-called fits in pigs. This is supported by the fact that two separate groups of fall pigs raised at the Minnesota Agricultural Experiment Station were later found to be partially or entirely depleted of vitamin D reserves at the time they were placed on experiments which are reported in this paper.

Many experiments have been conducted to determine the improvement in swine rations from additions of calcium, phosphorus, and vitamin D supplements. Unfortunately, it is not possible to interpret the results of the majority of these experiments in terms of the pig's requirement for vitamin D because in some the relationship between these mineral elements and vitamin D was not considered; and in others, the content of these nutrients in the control rations, as well as in the supplements, was not determined. It is of importance in nutrition experiments with swine, as well as in practical pig feeding, to know whether the pig is similar to the rat in not needing vitamin D when the calcium and phosphorus content of the ration is properly adjusted or whether it is similar to the chick in needing vitamin D regardless of the level of these mineral elements.

A series of investigations were conducted on the vitamin D requirement of the pig. It is the purpose of this paper to present data which show that the pig requires vitamin D and that there is considerable variation among breeds and even among pigs of the same breed in this requirement; that the reduced plasma-calcium values, as a result of lack of this vitamin, have a pronounced effect on growth and feed consumption; and that white pigs are less apt to become rachitic than colored pigs under similar conditions in the winter.

REVIEW OF LITERATURE

Bethke, Edgington, and Kick[2] found that there should be at least 0.60 percent of phosphorus in the ration of growing pigs in the absence of added vitamin D. A higher level, however, was not tested. With this percent of phosphorus, growth was satisfactory; and the calcium and inorganic phosphorus levels of the blood serum were not reduced, even though the calcium-phosphorus ratio varied from 1.2 to 3. Greater growth, however, resulted from the addition of cod-liver oil

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to a ration containing 0.45 percent of phosphorus. Apparently this means that the pig requires vitamin D and that a slight deficiency of the vitamin may affect growth without lowering the calcium and inorganic phosphorus levels of the blood. If the pig needs vitamin D, then there must have been storage of the vitamin during the preexperimental period in the pigs receiving the 0.60-percent phosphorus ration to which the vitamin had not been added. In fact, these investigators were of the opinion that the difference they found in ash values of femurs from fall-farrowed pigs as compared with those of spring-farrowed pigs was primarily due to variations in vitamin D storage.

The work of Sheehy[3] furnishes some evidence that the pig stores significant amounts of vitamin D. He concluded that there is considerable storage of the vitamin during the early fall months before the beginning of winter. Although his conclusion is probably correct, his experiments may also demonstrate that the pig requires little vitamin D in the presence of optimum amounts of calcium and phosphorus.

The experiments of Dunlop[4] likewise suggest storage of vitamin D by the pig. A ration containing 0.56 percent of phosphorus with a calcium-phosphorus ratio of 3 was satisfactory for summer but not for winter. In both cases the pigs were kept under confinement from the sun. Although he explained this difference as due to the greater amount of energy required to maintain the animal during winter than summer, it would seem that the difference may have been due to a greater storage of vitamin D by the pigs in the summer test before the start of the experiment than by those in the winter test. From several experiments on the pig's need for vitamin D with various levels of calcium and phosphorus, Dunlop concluded that the pig does not require added vitamin D providing the calcium and phosphorus levels are adequate as, for example, calcium levels of 0.47 or 0.86 percent and a phosphorus level of 0.56 percent. In view of a lack of information of vitamin D storage in his experimental pigs, it appears that such a broad statement as applying to all pigs in general is indeed questionable.

If there is a storage of vitamin D in the body of the pig as a result of solar irradiation or of feeding vitamin D supplements, sufficient to give protection from rickets during the usual experimental period, then the importance of a knowledge of such storage in vitamin D experiments is obvious.

Aubel and Hughes[5] recently reported that a ration containing 0.75 percent of phosphorus and having a calcium-phosphorus ratio of approximately 1 was decidedly unsatisfactory, even more so than a phosphorus level of 0.24 percent and a calcium-phosphorus ratio of 3. The best results were obtained from a ration containing 0.51 percent of phosphorus and a calcium-phosphorus ratio of 1.54. They believed that the results with the ration containing 0.75 percent of phosphorus were owing either to the high level of phosphorus or to the unfavorable calcium-phosphorus ratio. That neither of these explanations appears correct is indicated by the fact that Dunlop [6] obtained equally good results with a ration containing 0.53 percent of phosphorus and 0.86 percent of calcium as with a ration containing 0.95 percent of phosphorus and 0.79 percent of calcium. If pigs require vitamin D, then in the absence of the vitamin, rations higher in these mineral elements should be beneficial rather than detrimental. The most logical explanation for the performance of the pigs receiving the ration containing 0.75 percent of phosphorus is that the pigs needed vitamin D. This explanation is supported by the data showing low calcium of the blood serum.

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[5] AUBEL, C. E., and HUGHES, J. S. THE EFFECT ON GROWING PIGS OF RATIONS CONTAINING DIFFERENT LEVELS OF PHOSPHORUS IN THE ABSENCE OF VITAMIN D. Amer. Soc. Anim. Prod. 30: 334–340, illus. 1937.

It is evident that the literature does not permit a conclusion as to whether the pig needs vitamin D when receiving rations adequate in calcium and phosphorus, and, if there is such a need, whether it is of any practical significance.

EXPERIMENTAL PROCEDURE

FIRST EXPERIMENT

The first experiment, conducted from January 8 to March 12, 1934, involved three lots of five pigs per lot. In each lot there were one Hampshire, two Poland Chinas, and two Duroc-Jerseys. The preexperimental treatment of the Poland China pigs was not the same as for the other pigs, for the former had been obtained from the South East Minnesota Experiment Station at Waseca, Minn. All lots in this experiment, as well as in the experiments which follow, were housed in a heated swine barn. At no time did any of the pigs have access to sunshine or skyshine for the duration of the tests reported in this paper.

It was desired to use rations which were made up of natural foods to as great an extent as possible and which would give a high level of phosphorus. Corn and tankage were, therefore, used as the basal ingredients. Table 1 shows that a different kind of tankage was used for lot 1 than for lots 2 and 3. Previous tests had shown that tankage (2) had a much greater growth-promoting value than tankage (1). Since the need for vitamin D is dependent to a certain extent upon growth, it was felt that if the pigs required vitamin D, there might be a manifestation of this requirement sooner in lot 2 than in lot 1. The data which follow show that the need for vitamin D was so great that it completely overshadowed any difference in nutritive value of these supplements. The three rations were, therefore, essentially the same, with the exception that the rations for lots 1 and 2 did not contain added vitamin D while the ration for lot 3, used as the control, had an addition of irradiated yeast. Each ration, fed as a mixture, contained approximately 15 percent of protein, 0.65 of phosphorus, and 1.07 of calcium. This gave a calcium-phosphorus ratio of 1.65. Each group received the same quantity of feed.

In addition to the general condition and gross symptoms of the pigs, the level of calcium and inorganic phosphorus in the blood plasma was used to determine the need for vitamin D. The blood for these analyses was obtained from tail bleeding on 2 consecutive days.

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[6] DUNLOP, GEORGE. See footnote 4.

TABLE 1.—*Rations used in experiment 1 to determine the vitamin D requirement of the pig*

Ration 1		Ration 2		Ration 3	
Component	Percent	Component	Percent	Component	Percent
Yellow corn	86.00	Yellow corn	86 00	Yellow corn	86.00
Tankage (1)	11.50	Tankage (2)	12.11	Tankage (2)	12.11
Sodium chloride	.50	Sodium chloride	.50	Sodium chloride	.50
Yeast [1]	.10	Yeast [1]	.10	Irradiated yeast [1]	.10
Bonemeal	.96	Limestone	.59	Limestone	.59
Limestone	.16	Tapioca	.70	Tapioca	.70
Tapioca	.78				

[1] The yeast in all rations was increased to 0 15 percent on Feb. 14. The unirradiated yeast used was furnished by Standard Brands, Inc.

RESULTS

At the time the experiment was started, the Poland China pigs were quite unthrifty but were used because other pigs were not available. Two of the pigs in the group from which the six were taken for the experiment had had several tetanuslike convulsions. One of these pigs died during a convulsion, but an examination offered no explanation for its death or for the condition of the others.

However, the results of the blood-calcium analyses, made 1 week after the start of the experiment, readily explained their behavior. Table 2, in which all of the calcium and inorganic phosphorus determinations are given, shows that the Poland China pigs, Nos. 1, 2, 6, and 7, in lots 1 and 2 were suffering from slight to very great diminutions of blood calcium. The plasma calcium of one other pig in each of these lots, Nos. 5 and 9, respectively, was also low. The convulsions in the two pigs were undoubtedly a manifestation of low calcium tetany. The vitamin D stores in the pigs evidently had been depleted during the early part of the winter. Therefore, low-calcium tetany can be produced in pigs with a ration adequate in calcium but lacking in vitamin D or, as has since been reported by Hughes,[7] with a ration adequate in vitamin D but low in calcium. It is considered that the plasma calcium levels of the Poland China pigs, Nos. 11 and 12, in lot 3 were probably also low before the start of the experiment and that the vitamin D in the irradiated yeast had returned these values essential to normal. This statement is made in view of the similar initial unthrifty appearance of the Poland China pigs in all three lots.

During the course of the experiment, two other pigs, Nos. 4 and 7, were observed to have the tetanuslike convulsions. No. 7 had such severe convulsions that it was removed from the experiment on February 26. Four weeks after the start of the experiment pig No. 8 was dropped from the experiment because of a skin disease. Since it had been planned to feed all lots the same amounts of feed, the corresponding litter mates in the other lots were also dropped.

The data in table 2 show that, as the experiment progressed, the blood-plasma calcium of all of the pigs, with the exception of those in lot 3, decreased to very low levels. The change in the inorganic phosphorus, however, was relatively slight and highly variable. Four weeks after the first determinations, the plasma calcium of the pigs in lot 3 also decreased. Accordingly on February 14, the irradiated yeast was increased to 0.15 percent. That this was beneficial is evident from the higher plasma calcium values of lot 3 on February 27 and 28. A similar increase of the unirradiated yeast in the ration of lots 1 and 2 on February 14 was without benefit.

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[7] HUGHES, E. H THE CALCIUM AND INORGANIC PHOSPHORUS CONTENT OF THE BLOOD SERUM OF SWINE. Jour. Agr. Research 53: 267-279. 1936.

TABLE 2.—*Calcium and inorganic phosphorus in 100 cc of blood plasma of pigs at different times during experiment 1 to determine their vitamin D requirement*

Lot No	Pig No	Jan. 14-15		Feb. 11-12		Feb 27-28[1]		Mar. 11-12	
		Calcium	Inorganic phosphorus	Calcium	Inorganic phosphorus	Calcium	Inorganic phosphorus	Calcium	Inorganic phosphorus
		Milligrams	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
1	1	9.48	7.43	5.66	6.76	5.59	7.09	10.78	7.60
	2	6.94	7.49	5.97	7.02	5.23	7.41	-------	-------
	3	10.78	7.81	-------	-------	-------	-------	-------	-------
	4	10.57	8.55	8.85	7.87	5.59	7.94	11.08	7.81
	5	9.74	7.14	7.82	5.78	6.47	5.62	11.08	8.06
Mean		9.50	7.68	7.08	6.86	5.72	7.02	10.98	7.82
2	6	6.84	7.52	4.43	7.22	6.36	6.85	[2] 10.47	-------
	7	5.39	6.69	5.35	5.85	5.13	9.78	-------	-------
	8	10.16	7.52	-------	-------	-------	-------	-------	-------
	9	9.90	7.14	8.65	7.46	5.85	7.58	11.08	8.06
	10	10.16	7.07	9.37	6.60	7.08	8.55	10.93	7.43
Mean		8.49	7.19	6.95	6.78	6.11	8.19	[3] 11.01	7.75
3	11	10.68	7.63	9.57	7.14	9.75	8.37	9.70	7.04
	12	10.16	7.41	9.78	7.41	10.67	9.71	-------	-------
	13	11.30	6.92	-------	-------	-------	-------	-------	-------
	14	11.19	7.81	9.68	7.17	9.85	8.03	11.03	8.03
	15	10.57	6.54	10.09	7.14	10.67	8.30	10.88	7.81
Mean		10.78	7.26	9.78	7.22	10.24	8.60	10.54	7.63

[1] Nos. 2, 7, and 12 were bled on Feb 26.
[2] No 6 was bled on Mar. 4.
[3] Mean for Nos 9 and 10.

By February 28 all the pigs in lots 1 and 2 had severe cases of rickets, as judged from their low blood calcium. This was accompanied by harsh hair coats and an unthrifty appearance. On this date the unirradiated yeast in their ration was replaced by irradiated yeast. This had an immediate effect in raising the blood calcium, as seen in the normal value for pig No. 6 4 days after the change in yeast. The normal values for the remaining pigs 2 weeks after the change in the ration confirmed the result. During this period there was also an improvement in appetite.

The pigs in lots 1 and 2 had poor appetites and, consequently, made subnormal growth. During the 9 weeks of the experiment the pigs ate an average of only 2.69 pounds of feed daily. The feed consumption and growth records are given in table 3. The slow growth of all of the pigs in lot 3, except No. 14, can be explained by the fact that they were limited to the same feed intake as eaten by each of the other lots. It is felt that No. 14, being larger than the other pigs in the lot, was able to obtain more than its share of the feed.

TABLE 3.—*Growth and feed data of pigs at different times during experiment 1 to determine their vitamin D requirement*

Date	Weight of pigs in lot 1					Weight of pigs in lot 2					Weight of pigs in lot 3					
	No. 1	No 2	No 3	No 4	No. 5	No. 6	No 7	No. 8	No. 9	No. 10	No. 11	No. 12	No. 13	No. 14	No. 15	Average daily feed consumed per pig[1]
	Lb.	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb*	*Lb.*	*Lb*	*Lb.*	*Lb*	*Lb*	*Lb*	*Lb.*	*Lb.*
Jan. 8	39	42	62	47	57	40	40	59	47	57	33	35	68	56	51	------
Jan. 15	42	46	73	55	62	42	41	65	53	68	37	38	74	64	58	2.86
Jan. 22	45	47	77	60	63	45	39	67	56	73	36	40	79	72	61	2.17
Jan. 29	44	47	83	66	63	45	41	65	60	81	39	45	81	81	62	2.46
Feb. 5	44	51	95	75	72	48	40	67	70	90	40	50	86	91	67	2.31
Feb. 12	47	52	---	82	73	44	42	-----	79	98	41	54	----	97	74	2.68
Feb. 19	48	57	---	96	77	47	45	-----	87	107	46	60	-----	109	81	2.54
Feb. 26	53	61	-----	107	78	50	42	----	93	117	44	68	-----	119	90	2.86
Mar. 5	51	----	----	107	85	51	----	-----	95	120	50	----	-----	130	101	3.33
Mar. 12	57	----	-----	116	93	----	----	----	108	136	48	-----	-	145	112	3.38

[1] All lots were fed equal amounts.

SECOND EXPERIMENT

During the course of the first experiment the work of Dunlop[8] came to the authors' attention. He found that the pigs in his experiments required no vitamin D from weaning to bacon weight when receiving rations containing 0.56 percent of phosphorus and having calcium-phosphorus ratios of 0.83 to 1.54. The only explanation which appeared plausible for his finding as contrasted with that of the present writers was that the pigs in his experiments had stored sufficient vitamin D to protect them from rickets during the experimental period. This was suggested by the fact that the pigs used by Dunlop were of the Large White breed whereas in the writers' experiment only colored pigs were used. It was felt that there might be a greater storage of vitamin D by nonpigmented pigs than by those which are pigmented, and also that there might be an hereditary difference among breeds in their requirement for this vitamin.

Accordingly, two lots of pigs were placed on experiment December 12, 1935. Lot 1 contained one Duroc-Jersey, one Poland China, five Hampshires, and five Chester Whites. Lot 2, which received the control vitamin D-containing ration, consisted of two Poland Chinas and four Duroc-Jerseys. The initial weight of the Chester Whites was 50 pounds; of the Duroc-Jerseys and Poland Chinas, 55 pounds; and of the Hampshires, 75 pounds. They were individually fed twice daily.

The ration fed lot 1 consisted of yellow corn, 86.84 percent; tankage, 11.80 percent; sodium chloride, 0.50 percent; bonemeal, 0.26; limestone, 0.45 percent; and unirradiated yeast, 0.15 percent. The same ration was fed to lot 2 except irradiated yeast was used in place of the unirradiated yeast. The percentages of protein, calcium, and phosphorus in these rations were the same as those used in the first experiment.

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[8] DUNLOP, GEORGE. See footnote 4.

Since the first experiment showed that a depletion of vitamin D resulted in a marked change only in the calcium content of the blood plasma, only calcium analyses of the blood plasma from single blood samples were made in experiment 2.

RESULTS

The plasma-calcium analyses are shown in table 4. The reduction in plasma-calcium values, as a result of a lack of vitamin D, had other pronounced effects on the animals. The effects on growth and feed consumption, are presented for each pig for biweekly periods in tables 5 and 6.

TABLE 4.—*Calcium in 100 cc of blood plasma of pigs at different times during experiment 2 to determine the vitamin D requirement of different breeds of swine*

Lot No	Pig No.	Breed	Dec. 11	Jan. 9	Mar. 3	Mar 31
			Milligrams	*Milligrams*	*Milligrams*	*Milligrams*
1	1	Duroc-Jersey	9.26	10.14	6.91	
	2	Poland China	8.97	10.67	5.23	
	3	Hampshire	9.74	10.29	7.87	
	4	do	9.89	10.86	6.87	
	5	do	10.23	10.67	5.96	
	6	do	10.28	9.85	6.82	
	7	do	10.28	10.77	10.05	10.18
	8	Chester White	10.23	10.96	10.28	10.49
	9	do	9.79	10.86	10.65	10.23
	10	do	9.94	10.58	10.51	10.07
	11	do	9.79	10.58	9.37	8.22
	12	do	9.74	10.19	10.05	8.86
2	13	Poland China	9.31	10.09		
	14	do	9.70	10.82		
	15	Duroc-Jersey	9.21	9.90		
	16	do	9.26	10.29		
	17	do	9.01	10.43		
	18	do	9.89	10.62		

TABLE 5.—*Average daily gain in weight per pig at different times during experiment 2 to determine the vitamin D requirement of different breeds of swine*

Lot No.	Pig No.[1]	Dec 12–26	Dec 26–Jan 9	Jan 9–23	Jan 23–Feb 6	Feb 6–20	Feb. 20–Mar 6	Mar. 6–20	Mar. 20–Apr. 3
		Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
1	1	1.21	1.14	1.64	1.93	1.79	0.86		
	2	1.49	.93	1.36	1.49	1.21	[2] −.14		
	3	1.57	.86	1.07	1.71	1.29	[2] −.79		
	4	1.29	.93	1.79	1.71	1.93	.71		
	5	1.93	1.43	1.49	1.79	1.07	.86		
	6	2.14	1.07	.43	1.43	1.71	.43		
	7	2.07	1.21	1.93	1.49	1.29	1.49	2.57	1.86
	8	1.29	1.29	1.36	1.93	2.00	1.79	1.79	2.43
	9	1.79	1.14	1.93	1.79	2.14	1.71	1.49	1.93
	10	1.57	.86	1.71	1.93	1.36	1.57	1.64	1.57
	11	1.57	1.43	1.86	.64	2.21	1.43	.71	1.71
	12	1.29	.86	.93	.21	1.43	1.21	1.43	1.79
2	13	1.14	1.07	1.00	1.21	1.14	1.86	1.93	
	14	1.00	1.21	1.29	1.49	1.57	1.64	1.43	
	15	1.21	1.00	1.43	1.71	1.79	1.93	1.86	
	16	1.07	1.43	1.79	1.64	2.07	2.07	2.00	
	17	1.57	1.49	1.79	2.00	1.86	2.00	2.07	
	18	1.00	.79	1.43	1.07	1.21	1.57	2.07	

[1] See table 4 for breeds of the pigs.

[2] Represents average daily loss of weight.

TABLE 6.—*Average daily feed consumption per pig at different times during experiment 2 to determine the vitamin D requirement of different breeds of swine*

Lot No	Pig No.[1]	Dec. 12-26	Dec. 26-Jan. 9	Jan. 9-23	Jan. 23-Feb. 6	Feb. 6-20	Feb. 20-Mar. 6	Mar. 6-20	Mar. 20-Apr 3
		Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
1	1	2.48	3.32	4.41	5.28	6.04	5.66	4.80	4.80
	2	2.81	3.29	3.75	4.26	3.85	2.39	2.21	4.16
	3	3.69	3.73	4.15	5.48	5.37	1.75	4.46	--------
	4	4.69	4.76	5.51	5.64	6.52	5.75	2.73	--------
	5	3.74	4.65	5.16	5.83	5.14	4.37	4.09	4.70
	6	4.31	4.05	4.18	5.09	5.39	4.73	4.36	5.08
	7	4.46	5.46	6.34	6.53	7.93	7.50	8.49	8.64
	8	3.00	3.74	4.09	5.13	6.19	6.51	7.19	8.65
	9	3.16	4.05	5.23	5.77	6.38	6.99	7.55	8.04
	10	3.02	3.50	4.44	5.21	5.34	6.06	6.57	7.70
	11	3.14	4.16	5.03	5.76	6.61	6.69	4.49	7.45
	12	2.51	3.09	3.08	3.39	4.26	4.65	5.82	6.27
2	13	2.09	3.14	3.23	3.51	3.86	4.84	6.34	--------
	14	2.41	3.46	4.00	4.11	4.96	5.22	6.44	--------
	15	2.92	3.51	4.78	5.06	5.91	7.18	8.11	--------
	16	2.79	4.34	5.19	5.22	5.57	7.18	8.07	--------
	17	2.79	4.34	5.19	5.22	6.57	6.62	7.29	--------
	18	1.85	2.73	3.75	4.25	5.34	5.59	7.34	--------

[1] See table 4 for breeds of the pigs.

The fact that 14 of the 18 pigs showed less than 10 mg of calcium per 100 cc of blood plasma on December 11, before the experiment was started, suggested that these pigs were becoming rachitic. However, 4 weeks later, when the next determinations were made, there had been a significant increase in the calcium content of the blood plasma of all of these pigs. Therefore, it seems probable that the low plasma-calcium levels on the earlier date were the result of rations inadequate in calcium. As the experiment progressed, it was apparent that several of the pigs in lot 1, Nos. 2, 3, 4, 5 and 6, were becoming rachitic. This was indicated by their lameness, loss of appetite, and slow growth or even loss in weight. Pigs Nos. 2, 3, and 4 also had several convulsions. Plasma-calcium determinations on some of these pigs revealed that the calcium of the blood was gradually decreasing. Therefore, on March 3, calcium analyses were made of the blood plasma of all the pigs in lot 1. Similar analyses were not made for lot 2 because they were receiving 0.15 percent of irradiated yeast, which, in the first experiment, not only protected the pigs from rickets but also cured severe rickets as well. The results of these analyses indicate that the Duroc-Jersey, Poland China, and the Hampshires, Nos. 3 to 6, inclusive, had very low plasma-calcium values. The records of feed intake and growth show the effects of their rachitic condition. In their behavior and physical appearance they resembled the rachitic pigs in the first experiment. With the exception of Chester White pig No. 11, all the pigs of this breed and Hampshire pig No. 7 had normal values for plasma calcium. When calcium analyses of the blood plasma were again made on March 31, 110 days after the start of the experiment, only Chester White pigs No. 12 and No. 11 showed a reduction in plasma calcium. However, growth and feed intake of these two pigs were satisfactory for the period from March 20 to April 3.

The experiment was terminated on April 3 because facilities were no longer available for continuing it. Had the pigs which were not at this time rachitic been continued on the diet for a longer period, it is believed that they would have eventually become so. That it was probably a question of greater storage of vitamin D by the white pigs as compared with those which were pigmented is supported by the fact that pig No. 7, the only Hampshire which did not become rachitic, was almost entirely white.

THIRD EXPERIMENT

In view of the results of the second experiment, it was desired to investigate further the difference in the vitamin D requirement of white and colored pigs. For this purpose two lots of six pigs each, each pig averaging approximately 45 pounds in weight, were started on experiment December 21, 1936. Lot 1 was made up of six Chester White pigs and lot 2 of three Duroc-Jerseys and three Poland Chinas. The previous treatment of all the pigs had been the same. Neither lot was allowed access to sunlight. The ration self-fed to both lots consisted of corn, 86 percent; tankage, 12 percent; salt, 0.50 percent; bonemeal, 1.0 percent; and limestone, 0.50 percent. The calcium, phosphorus, and protein in the ration were 1.05, 0.63, and 16.60 percent, respectively. Individual weights of the pigs and the feed eaten by each group were recorded at 2-week intervals. As in the second experiment, the calcium content of the blood plasma of the pigs was used as the index of the need for vitamin D. The calcium, growth, and feed data are shown in tables 7- 8, and 9, respectively.

TABLE 7.—*Calcium in 100 cc of blood plasma of pigs at different times during experiment 3 to determine the vitamin D requirement of white and colored animals*

Lot No	Pig No	Breed	Dec. 21	Jan. 18	Feb. 2	Feb. 8	Feb 15	Mar. 2
			Milli-grams	*Milli-grams*	*Milli-grams*	*Milli-grams*	*Milli-grams*	*Milli-grams*
1	1	Chester White	11.64	12.36			9.72	9.77
	2	do	10.58	11.02			9.67	9.11
	3	do	10.53	11.56			10.59	9.72
	4	do	11.59	11.61			10.13	9.06
	5	do	11.27	11.34			9.57	8.24
	6	do	10.95	11.08			9.46	9.16
Mean			11.09	11.50			9.86	9.18
2	7	Duroc-Jersey	10.05	9.24	8.59	8.23	6.96	
	8	do	10.95	9.26	7.76	7.24	6.65	
	9	do	10.69	10.27	8.23	7.40	6.92	
	10	Poland China	11.64	10.64	9.31	8.90	7.42	
	11	do	11.01	9.63	8.12	7.81	7.37	
	12	do	11.33	9.84	7.76	6.42	6.04	
Mean			10.95	9.81	8.30	7.67	6.89	

TABLE 8.—*Average daily gain in weight per pig at different times during experiment 3 to determine the vitamin D requirement of white and colored animals*

Lot No.	Pig No.[1]	Dec. 21-Jan. 4	Jan. 4-18	Jan. 18-Feb. 1	Feb. 1-15	Feb. 15-Mar. 1
		Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*
1	1	1.00	1 07	0.79	1.14	1.36
	1	1.00	1.00	1.21	1.36	1.50
	3	1.29	1.21	1.43	1.57	.71
	4	1.43	1.07	1.14	1.14	1.43
	5	1.50	1.21	1.50	1.79	1 93
	6	.93	.64	1.14	.86	1 00
Mean		1 19	1.03	1.20	1.31	1 32
2	7	29	.36	.29	.64	
	8	79	29	.50	.79	
	9	1.29	1.21	1.00	.86	
	10	1 14	1.43	1.07	1 50	
	11	1.21	93	1.00	.57	
	12	.79	.71	.43	29	
Mean		.92	.82	.72	.78	

[1] See table 7 for breeds of the pigs.

TABLE 9.—*Average daily feed consumed per pig at different times during experiment 3 to determine the vitamin D requirement of white and colored animals*

Lot No.	Dec 21-Jan 4	Jan. 4-Jan. 18	Jan 18-Feb. 1	Feb 1-Feb 15	Feb 15-Mar. 1
	Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*
1	2.68	3 16	3 56	4 17	4 45
2	2 61	3 05	3 24	3 23	

RESULTS

The data in table 7 show that 28 days after the start of the third experiment the blood-calcium value for each of the Chester White pigs was slightly higher than when the pigs were put on the experiment. After the test had been in progress for 56 days, there was a reduction from the former average value of 11.5 mg to 9.86 mg; 2 weeks later there was a further reduction to 9.18 mg. At this time all of the pigs were quite sore-footed. Some were buckling over on the front pasterns and were becoming bowlegged, both in front and behind.

Table 7 reveals quite a different picture for the Duroc-Jersey and Poland China pigs in lot 2. This table shows that it required twice as long for the blood calcium of the Chester White pigs to decrease to essentially the same level as that of the colored pigs. Two of the possible explanations of this result are as follows: Either there was a greater storage of vitamin D in the Chester White pigs or their requirement for the vitamin was less. If it can be assumed that similar reduced levels of blood calcium in the two groups of pigs is indicative of similar residual stores of vitamin D, then the requirement of the Chester White pigs for the vitamin was actually less, as evidenced by the slower rate of decrease of their blood calcium. Some variability also existed in this respect among pigs of the same breed in lot 2. In another study of the vitamin D requirement of growing pigs, two Duroc-Jersey pigs, litter mates, did not become rachitic until 97 days after the start of the experiment. Ten other pigs, which had been under a similar preexperimental environment, became rachitic in 28 days. This suggests that there may be important hereditary differences in the vitamin D requirement of pigs, as Gowen[9] found to be the case with rats.

There was a gradual reduction in blood calcium of the Duroc-Jersey and Poland China pigs to 6.89 mg. Some of these pigs were lame, but this condition was not characteristic of the entire group. It was clearly evident from the blood picture and from the records on growth and feed intake, tables 8 and 9, that these pigs were rachitic and were reaching a level of blood calcium where convulsions could be expected. Both lots were, therefore, exposed to the sun, the exposure bringing about complete recovery.

The results of the third experiment confirm the results of the second experiment showing that white pigs are much less apt to become rachitic under confinement during the winter-feeding period than colored pigs. The explanation for the fact that the white pigs in the third experiment became rachitic in 56 days while most of the white pigs in the second experiment had not become rachitic even after 110 days is that the pigs in the earlier experiment had probably received more vitamin D either through solar irradiation or their feed before the start of the test. This is further supported by the fact that it likewise required longer for the colored pigs in the second test to become rachitic than in the third test.

SUMMARY AND CONCLUSIONS

The growing pig's requirement for vitamin D was investigated during the winters of 1935, 1936, and 1937 with fall-farrowed pigs of the Chester White, Duroc-Jersey, Hampshire, and Poland China breeds of swine. The basal ration used in the experiments contained approximately 0.64 percent of phosphorus and a calcium-phosphorus ratio of approximately 1.65.

The results of these tests showed conclusively that the pigs required added vitamin D after the body stores of the vitamin had been depleted. With the exception of two Duroc-Jersey pigs, all of the colored pigs, having access to the outside during the fall before being placed under confinement, stored sufficient vitamin D to protect them from rickets for periods of only 4 to 8 weeks. White pigs, of the Chester White breed and Hampshire pig No. 7, which was nearly white, raised under the same environment as the colored pigs, were protected for approximately twice as long.

A depletion of vitamin D resulted in a reduction of the blood-plasma calcium from between 10 and 12 mg. per 100 cc. of blood plasma to below 6 mg., the extent of reduction depending on the length of time the pigs were kept on the vitamin D-free ration. When low blood-calcium values were reached, calcium-deficiency tetany was manifested. The reductions in blood calcium were accompanied by a gradual loss of appetite and an unthrifty appearance, characterized by emaciation, a rough hair coat and, usually but not always, lameness. An almost complete loss of appetite invariably resulted with the onset of low-calcium tetany.

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[9] GOWEN, JOHN. INHERITANCE AS IT AFFECTS SURVIVAL OF RATS FED A DIET DEFICIENT IN VITAMIN D. Genetics 21: 1-23, illus. 1936.

BACTERICIDAL EFFICIENCY OF CERTAIN ANILINE DYES[1]

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INTRODUCTION

During the last few years the writer has had occasion to make a considerable number of tests of the bactericidal efficiency of various aniline dyes. The ultimate object was to ascertain the utility of these dyes in the preparation and preservation of biological products to be used for the prophylaxis or diagnosis of animal diseases. The biological products particularly involved were hog cholera virus, hog cholera vaccine, and stained antigens for the detection of pullorum disease in fowls and Bang's disease in cattle. Accordingly, use was made of *Escherichia coli*, *Salmonella pullorum*, and *S. suipestifer* as test organisms in addition to *Staphylococcus aureus* and *Eberthella typhi*, which are more commonly used in testing disinfectants. With the exception of *Staphylococcus aureus*, all the test organisms were Gram-negative. The present paper reports the results of representative bactericidal tests of readily available dyes, made in order to select those most suitable for experiments with the individual biological products.

EXPERIMENTS WITH AQUEOUS SOLUTIONS

The initial experiments were conducted on solutions of dyes to which no extraneous organic matter had been added other than that introduced with the cultures of micro-organisms. Bactericidal efficiency was determined by the Rideal-Walker technique, modified as follows: Beef-infusion broth adjusted to pH 7.2 was used instead of R-W standard broth; a 1-cc pipette graduated into tenths was employed for measuring the 0.5 cc of culture required for the test; the temperature at which the experiments were conducted was 25° C. instead of 15° to 18°; dilutions were made in sterile Erlenmeyer flasks instead of in cylinders; fixed quantities of the stock solution of disinfectant were added to varying quantities of water, both being measured by sterile standardized pipettes; several test organisms were used in addition to *Eberthella typhi* (*Bacillus typhosus*); the total time of exposure was increased from 15 minutes to 60 minutes; finally, no phenol coefficients were calculated, bactericidal efficiency being measured by the concentration of disinfectant required to kill the test organism. No attempt was made to neutralize dye carried over into subcultures, but whenever it seemed necessary secondary subcultures were made from the original subcultures.

In table 1 are shown representative results obtained with various commercial dyes in aqueous solution. In those instances in which the dye content was known it is stated, but the concentrations given in the table are based on total weight of the commercial dye without regard to the percentage of actual dye therein.

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[1] Received for publication November 25, 1938.

TABLE 1.—*Bactericidal efficiencies of various commercial aniline dyes in aqueous solution*[1]

Dye	Dilution of dye[2]	Results after exposure for[3]— 5 minutes	10 minutes	15 minutes	30 minutes	45 minutes	60 minutes	Test organism
Auramine 0	1-200	−	−	−	−	−	−	*Eberthella typhi.*
	1-400	+	+	+	−	−	−	
	1-600	+	+	+	+	+	+	
	1-400	+	−	−	−	−	−	*Salmonella suipestifer*
	1-600	+	+	−	−	−	−	
	1-800	+	+	+	−	−	−	
	1-1,000	+	+	+	+	−	−	
	Saturated solution	+	+	+	+	+	+	*Staphylococcus aureus*
Basic fuchsin (92 percent dye).	1-400	+	+	+	−	−	−	*Escherichia coli*
	1-600	+	+	+	+	−	−	
	1-800	+	+	+	+	+	+	
	1-400	+	+	−	−	−	−	*Salmonella suipestifer*
	1-600	+	+	+	−	−	−	
	1-800	+	+	+	+	−	−	
	1-1,000	+	+	+	+	+	+	
	1-400	−	−	−	−	−	−	*Staphylococcus aureus*
	1-600	+	+	+	+	−	−	
Malachite green (76-percent dye)	1-1,200	−	−	−	−	−	−	*Eberthella typhi.*
	1-1,600	+	+	−	−	−	−	
	1-2,000	+	+	+	+	+	−	
	1-800	+	+	−	−	−	−	*Escherichia coli*
	1-1,000	+	+	+	+	+	+	
	1-1,200	+	−	−	−	−	−	*Salmonella suipestifer*
	1-1,600	+	+	−	−	−	−	
	1-2,000	+	+	+	+	+	+	
	1-4,000	−	−	−	−	−	−	*Staphylococcus aureus*
	1-8,000	+	+	+	+	+	+	
Brilliant green (94-percent dye)	1-1,200	+	+	−	−	−	−	*Escherichia coli.*
	1-1,400	+	+	+	+	+	+	
	1-1,200	−	−	−	−	−	−	*Salmonella suipestifer.*
	1-1,400	+	+	−	−	−	−	
	1-1,600	+	+	+	+	+	−	
	1-20,000	+	+	−	−	−	−	*Staphylococcus aureus*
	1-21,000	+	+	+	+	+	−	
Hofmann's violet	1-200	+	+	+	+	+	+	*Escherichia coli*
	1-400	+	+	−	−	−	−	*Salmonella suipestifer*
	1-800	+	+	+	−	−	−	
	1-1,200	+	+	+	+	+	−	
	1-2,000	+	+	+	−	−	−	*Staphylococcus aureus.*
	1-4,000	+	+	+	+	+	+	
Methyl violet 2B (81-percent dye)	1-2,000	+	+	+	+	−	−	*Eberthella typhi.*
	1-3,000	+	+	+	+	+	+	
	1-200	+	+	+	−	−	−	*Escherichia coli*
	1-400	+	+	+	+	+	−	
	1-1,200	+	+	−	−	−	−	*Salmonella suipestifer*
	1-1,600	+	+	+	+	+	+	
	1-2,000	−	−	−	−	−	−	*Staphylococcus aureus.*
	1-3,000	+	+	+	−	−	−	
	1-4,000	+	+	+	+	+	+	
Crystal violet (88-percent dye).	1-1,000	+	+	−	−	−	−	*Eberthella typhi.*
	1-2,000	+	+	+	+	+	+	
	1-100	+	+	+	−	−	−	*Escherichia coli.*
	1-200	+	+	+	+	+	+	
	1-500	+	+	+	+	+	−	*Salmonella pullorum.*
	1-750	+	+	+	+	+	+	
	1 500	+	+	+	+	+	−	*Salmonella suipestifer.*
	1-750	+	+	+	+	+	+	
	1-3,000	+	+	−	−	−	−	*Staphylococcus aureus.*
	1-4,000	+	+	+	+	+	+	
Victoria blue	1-200	+	+	+	−	−	−	*Salmonella suipestiter.*
	1-400	+	+	+	+	+	+	
	1-4,000	+	−	−	−	−	−	*Staphylococcus aureus.*
	1-6,000	+	+	+	+	−	−	

[1] Experiments conducted at 25° C.
[2] On basis of commercial dye.
[3] Growth, (+); no growth, (−).

All the dyes shown in table 1 are basic dyes and, with the exception of auramine 0, a diphenylmethane compound, all are triphenylmethane derivatives. Basic fuchsin, a simple triamino derivative, showed comparatively low bactericidal activity, which was approximately the same against Gram-positive and Gram-negative organisms. All the other dyes carry varying heavy substitutions in the amino groups. Except auramine 0, all manifested much greater bactericidal activity than basic fuchsin and much greater activity against Gram-positive than against Gram-negative organisms. Auramine 0 is noteworthy as an exception to the usually accepted rule that basic dyes are more effective against Gram-positive than against Gram-negative organisms.

Additional data, not presented in table 1 but obtained in the same manner, are as follows: Ethylated crystal violet (ethyl green) was less than one-tenth as efficient as crystal violet itself, and ethyl violet, a homologue of crystal violet, was entirely ineffective in saturated solution; sulphonated brilliant green (light green SF yellowish) was ineffective in 20-percent solution; the thiazine dyes thionine and methylene blue were ineffective, the former in saturated solution and the latter in 2-percent solution; the disazo dye Bismarck brown was ineffective in 1-percent solution.

In table 2 are shown results of experiments in which phenol or orthocresol was added to solutions of some of the same dyes. As in table 1, dye concentration is based on total weight of commercial dye.

TABLE 2.—*Influence of phenol or orthocresol on bactericidal efficiencies of dyes* [1]

Dye	Dilution of dye [2]	Results after exposure for [3] —						Test organism
		5 minutes	10 minutes	15 minutes	30 minutes	45 minutes	60 minutes	
Basic fuchsin used with Phenol (1-1,000)	1-400	+	+	−	−	−	−	*Escherichia coli.*
	1-600	+	+	+	−	−	−	
	1-800	+	+	+	+	+	+	
	1-700	+	+	−	−	−	−	*Salmonella suipestifer*
	1-800	+	+	+	−	−	−	
	1-900	+	+	+	+	−	−	
Malachite green used with—								
Phenol (1-1,000)	1-1,200	+	+	+	−	−	−	*Escherichia coli*
	1-1,400	+	+	+	+	+	+	
Orthocresol (1-1,000)	1-2,000	+	+	+	−	−	−	
	1-2,400	+	+	+	+	+	+	
Brilliant green used with—								
Phenol (1-1,000)	1-1,200	−	−	−	−	−	−	*Escherichia coli*
	1-1,600	+	+	+	+	+	+	
Orthocresol (1-1,000)	1-1,600	+	+	−	−	−	−	
	1-2,000	+	+	+	+	+	+	
Phenol (1-1,000)	1-1,400	+	−	−	−	−	−	*Salmonella suipestifer*
	1-1,600	+	+	+	+	+	+	
	1-16,000	+	+	+	+	−	−	*Staphylococcus aureus*
	1-20,000	+	+	+	+	+	−	
	1-24,000	+	+	+	+	+	+	
Orthocresol (1-1,000)	1-18,000	+	+	−	−	−	−	
	1-24,000	+	+	+	+	+	+	
Methyl violet 2B used with—								
Phenol (1-1,000)	1-2,000	+	−	−	−	−	−	*Eberthella typhi*
	1-4,000	+	+	+	+	+	+	
Orthocresol (1-1,000)	1-4,000	−	−	−	−	-	−	
	1-6,000	+	+	+	+	+	+	
Phenol (1-1,000)	1-800	+	−	−	−	−	−	*Escherichia coli.*
	1-1,600	+	+	+	+	−	−	
	1-2,400	+	+	+	+	+	+	
Orthocresol (1-1,000)	1-3,000	+	−	−	−	−	−	
	1-4,000	+	+	+	−	−	−	
	1-5,000	+	+	+	+	+	−	
Phenol (1-1,000)	1-2,000	+	+	−	−	−	−	*Salmonella suipestifer.*
	1-3,000	+	+	+	−	−	−	
	1-4,000	+	+	+	+	−	−	
	1-5,000	+	+	+	+	+	−	
Orthocresol (1-1,000)	1-4,000	+	+	−	−	−	−	
	1-5,000	+	+	+	−	−	−	*Staphylococcus aureus.*
Phenol (1-1,000)	1-3,000	+	−	−	−	−	−	
	1-4,000	+	+	+	+	+	+	

See footnotes at end of table.

TABLE 2.—*Influence of phenol or orthocresol on bactericidal efficiencies of dyes*—Continued

Dye	Dilution of dye	Results[3] after exposure for—						Test organism
		5 minutes	10 minutes	15 minutes	30 minutes	45 minutes	60 minutes	
Crystal violet used with—								
Phenol (1-1,000)	1-2,000	+	–	–	–	–	–	*Eberthella typhi*
	1-4,000	+	+	–	–	–	–	
	1-6,000	+	+	+	+	+	+	
	1-2,000	+	+	+	–	–	–	*Escherichia coli*
	1-3,000	+	+	+	+	–	–	
	1-4,000	+	+	+	+	+	+	
Phenol (equal weight)	1-500	–	–	–	–	–	–	
	1-1,000	+	–	–	–	–	–	
	1-1,500	+	+	+	+	+	–	
Phenol (preformed addition compound)[4]	1-730	–	–	–	–	–	–	
	1-1,460	+	+	+	+	–	–	
	1-2,190	+	+	+	+	+	+	
Orthocresol (1-1,000)	1-4,000	+	–	–	–	–	–	
	1-6,000	+	+	–	–	–	–	
	1-8,000	+	+	+	–	–	–	
Phenol (1-1,000)	1-1,000	–	–	–	–	–	–	*Salmonella pullorum.*
	1-2,000	+	+	+	+	+	–	
	1-2,000	+	–	–	–	–	–	*Salmonella suipestifer*
	1-3,000	+	+	+	+	+	–	
Orthocresol (1-1,000)	1-4,000	+	–	–	–	–	–	
	1-8,000	+	+	+	–	–	–	
	1-12,000	+	+	+	+	+	+	
Phenol (1-1,000)	1-2,000	–	–	–	–	–	–	*Staphylococcus aureus.*
	1-4,000	+	+	+	+	+	+	
Orthocresol (1-1,000)	1-2,000	–	–	–	–	–	–	
	1-4,000	+	+	+	–	–	–	
	1-6,000	+	+	+	+	+	+	

[1] Experiments conducted at 25° C.
[2] On basis of commercial dye, actual purity shown in table 1.
[3] Growth (+), no growth (–).
[4] The addition compound contains 68.5 percent of crystal violet and 31.5 percent of phenol.

Comparison of data in tables 1 and 2 indicates that the presence of phenol or orthocresol had little or no effect on the efficiency of triphenylmethane dyes against the Gram-positive *Staphylococcus aureus* but a varying favorable effect on their efficiency against Gram-negative organisms. The most notable effect was in the action of crystal violet on *Escherichia coli.* This increased efficiency is presumably due to the formation of addition compounds, described by Holmes and Hann,[2] who state, without presenting experimental data, that such compounds are germicidally active. In the case of crystal violet and phenol, the data in table 2 indicate that it was immaterial whether solutions of the two substances were merely mixed before use or whether the addition compound, containing 2 molecules of phenol to 1 molecule of crystal violet, was separated from admixed concentrated solutions, washed, and redissolved.

In table 3 are shown the effects, on the bactericidal powers of solutions of crystal violet, of the addition of sodium borate, sodium carbonate, or disodium phosphate. In this table, unlike tables 1 and 2, the dye concentration represents the actual dye content of the solution.

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[2] HOLMES, W. C., and HANN, R. M. REACTIONS OF BASIC DYES WITH CYCLIC DERIVATIVES OF AN ACID CHARACTER. Stain Technol. 3: 122-130. 1928.

TABLE 3.—*Influence of alkaline salts on the bactericidal efficiency of crystal violet* [1]

Alkaline salt added (1-1,000)	Dilution of dye [2]	Results after exposure for [3]						Test organism
		5 minutes	10 minutes	15 minutes	30 minutes	45 minutes	60 minutes	
None	1-1,000	+	−	−	−	−	−	*Eberthella typhi.*
	1-2,000	+	+	−	−	−	−	
	1-4,000	+	+	+	+	+	+	
Na_2CO_3	1-2,000	+	−	−	−	−	−	
	1-4,000	+	+	−	−	−	−	
	1-6,000	+	+	+	−	−	−	
None	1-200	+	+	+	−	−	−	*Escherichia coli*
	1-400	+	+	+	+	+	−	
Na_2CO_3	1-1,000	+	+	−	−	−	−	
	1-2,000	+	+	+	−	−	−	
	1-4,000	+	+	+	+	−	−	
	1-6,000	+	+	+	+	+	−	
	1-8,000	+	+	+	+	+	+	
None	1-500	+	+	+	−	−	−	*Salmonella pullorum*
	1-1,000	+	+	+	+	+	+	
Na_2CO_3	1-4,000	+	+	−	−	−	−	
	1,-8000	+	+	+	+	−	−	
	1-12,000	+	+	+	+	+	+	
None	1-400	+	−	−	−	−	−	*Salmonella suipestifer*
	1-600	+	+	+	−	−	−	
	1-800	+	+	+	+	+	−	
Na_2CO_3	1-1,000	+	+	−	−	−	−	
	1-2,000	+	+	+	−	−	−	
	1-4,000	+	+	+	+	+	+	
Na_2HPO_4	1-500	+	+	−	−	−	−	
	1-1,000	+	+	+	+	−	-	
	1-2,000	+	+	+	+	+	+	
$Na_2B_4O_7$	1-500	+	+	−	−	−	−	
	1-1,000	+	+	+	+	−	−	
	1 2,000	+	+	+	+	+	+	
None	1-4,000	−	−	−	−	−	−	*Staphylococcus aureus*
	1-6,000	+	+	+	+	+	+	
Na_2CO_2	1-6,000	−	−	−	−	−	−	
	1-8,000	+	+	+	+	+	+	

[1] Experiments conducted at 25° C

[2] On basis of true dye content, actual purity shown in table 1.

[3] Growth, (+), no growth, (−)

The results shown in table 3 indicate that the addition of sodium carbonate to solutions of crystal violet produced a marked increase in bactericidal efficiency, whereas sodium borate and disodium phosphate had comparatively little effect. The pH values of the mixtures containing sodium carbonate were slightly above 10, and of the mixtures containing sodium borate and disodium phosphate the pH values were between 8 and 9. When solutions containing sodium carbonate and crystal violet were allowed to stand several hours before being tested, some of the dye was precipitated and bactericidal efficiency diminished though still remaining greater than the efficiency of the dye alone.

EXPERIMENTS WITH CONTAMINATED HOG BLOOD

On the basis of the experiments with aqueous solutions, crystal violet was selected as the most promising dye for tests of bactericidal efficiency in the presence of a high concentration of defibrinated hog blood. Cultures of *Staphylococcus aureus*, *Salmonella suipestifer*, and *Escherichia coli* were mixed and added to the blood. After the addition of strong solutions of the bactericide, the mixtures were placed in an incubator at 37.5° C., and subcultures were made after 3, 7, and 14 days. Obviously, such tests are subject to considerable variation when attempts are made at duplication. Consequently, the data from a considerable number of experiments are only briefly summarized, as follows:

Mixtures containing phenol and crystal violet in concentrations of 1–500 and 1–2,000, respectively, and mixtures containing orthocresol and crystal violet in concentrations of 1–1,000 and 1–2,000, respectively, were sometimes effective and sometimes not. But mixtures containing orthocresol in a concentration of 1–500 and crystal violet in a concentration of 1–2,000 were uniformly effective even in the presence of very heavy bacterial contamination. Both phenol and orthocresol produced an undesirable thickening of the mixtures containing them. The admixture of sodium carbonate and crystal violet in final concentrations of 0.02 molar and 1–2,000, respectively, failed to kill the test organisms. Under like conditions, mixtures containing disodium phosphate were usually effective and mixtures containing sodium borate were uniformly effective. In these experiments the pH values of hog-blood mixtures containing sodium carbonate varied from 7.9 to 8.5; of those containing sodium borate, from 8.0 to 8.5; and of those containing disodium phosphate, from 7.3 to 7.4. Both sodium carbonate and sodium borate produced undesirable changes in the appearance and consistency of the mixtures, whereas disodium phosphate did not.

On the basis of the results obtained, the mixture of crystal violet and disodium phosphate was recommended as most suitable for the preparation of vaccine against hog cholera.

SUMMARY

Experimental information is presented regarding the bactericidal efficiency of several aniline dyes against *Eberthella typhi*, *Escherichia coli*, *Salmonella pullorum*, *Salmonella suipestifer*, and *Staphylococcus aureus*. Data presented indicate that the addition of phenol or orthocresol to solutions of crystal violet or methyl violet 2B greatly increases their bactericidal efficiency against Gram-negative bacteria. There is little or no increase in efficiency against Gram-positive bacteria. The addition of phenol or orthocresol to solutions of brilliant green or malachite green has little or no effect on their bactericidal efficiency.

The addition of sodium carbonate to aqueous solutions of crystal violet markedly increases bactericidal efficiency. The addition of sodium borate or disodium phosphate to such solutions produces little or no effect. In contaminated hog-blood mixtures kept at incubator temperature, crystal violet (1–2,000) with 0.02 molar sodium carbonate appears to be uniformly ineffective; with 0.02 molar disodium phosphate, usually effective; and with 0.02 molar sodium borate, uniformly effective, in killing the contaminating bacteria.

IDENTITY AND PARASITISM OF A SPECIES OF DOTHICHLOË[1]

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INTRODUCTION

Among the fungi that cause sterility diseases of certain grasses is an undescribed species of *Dothichloë* which has engaged the writer's attention intermittently for some 10 years. Its specific identity has not hitherto been distinguished, but in the literature it has been confused with related organisms so that an interest in the fungus itself is no less justifiable than in its rather remarkable pathological effects.

This fungus is rarely noted by the casual observer, although to one who has once become familiar with it the fructifications are evident first as white, then as black areas up to 2 cm in diameter on the upper (adaxial) leaf surfaces of infected grasses (pl. 1, *A* and *B*). The white or conidial stage is relatively conspicuous only under moist conditions when the grass leaves are distended, but the matured black ascostromata stand out plainly, being especially noticeable when, in response to drying, the partially inrolled leaf blades of the host plants fail to roll up at the normal rate in the stroma-bearing areas. Shortly after maturity, however, this black fungus layer sloughs off, restoring the leaf surface to a nearly normal coloration.

In a previous publication[3] on the life history of this fungus it was referred to as *Dothichloë atramentosa* Atk. Later studies, including comparisons with pertinent fungi exsiccati and reexamination of the literature, have verified the conclusions there recorded but have compelled the recognition of the fungus as a distinct species and have revealed considerable additional detail thought worthy of record.

GEOGRAPHIC DISTRIBUTION

In the southeastern part of the United States the fungus here discussed occurs commonly on *Sporobolus poiretii* (Roem. and Schult.) Hitchc. as a sterilizing parasite perhaps second in importance only to the ubiquitous *Helminthosporium ravenelii* Curt., and it has been found on at least nine other grass host species distributed among eight chiefly unrelated genera, not only in the Southeastern States but also in North Dakota and in Brazil, Surinam, and the West Indies.

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[1] Received for publication November 4, 1938 Contribution No 168 from the laboratories of Cryptogamic Botany and the Farlow Herbarium, Harvard University. The material presented here is the result of work utilizing certain facilities provided by Harvard University in cooperation with the U S Department of Agriculture.

[2] The writer is indebted to J. L. Seal, Alabama Agricultural Experiment Station, and R. W Davidson, Division of Forest Pathology, Bureau of Plant Industry, U. S Department of Agriculture, for certain culture materials from Alabama and Louisiana, respectively, to E. F. Guba, Massachusetts Agricultural Experiment Station, for the use in 1930 and 1931 of greenhouse space at the Waltham Field Station; and particularly to W. H. Weston, professor of cryptogamic botany, Harvard University, for sustained interest and helpful criticism. To the many persons who have aided through the contribution of specimens it is impossible to express individual acknowledgment, but for permission to examine specimens in their charge and for incidental courtesies thanks are especially due to the following: F J Seaver, New York Botanical Garden; Erdman West, Florida Agricultural Experiment Station; and C. W. Dodge and D. H. Linder, Farlow Herbarium of Harvard University.

[3] DIEHL, WILLIAM W. CONIDIAL FRUCTIFICATIONS IN BALANSIA AND DOTHICHLOË Jour. Agr. Research 41: 761-766, illus 1930.

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TAXONOMY

Although this organism already has a plethora of names, none of them is suitable because all are technically invalid. It seems necessary, therefore, to assign it a new name in order to prevent further confusion.

SYNONYMY

Dothichloë limitata n. sp.

Sphaeria epichloè Kze., 1827, nom. in sched. in Weigelt's exs. (not numbered), not *Botryosphaeria epichloè* Sacc., 1892, Fung. Herb. Brux. No. 11.

Dothidea atramentaria Berk. and Curt., 1876, nom. nud., Grevillea 4: 105; in Rav. F. Am. exs. No. 100, 1878; and in Ell. N. Am. F. No. 683, 1881.

Dothidea atramentaria Berk. and Curt. f. *eragrostidis-capillaris* Thüm., 1879, nom. nud., in DeThüm, Myc. Univ. No. 1068.

Ophiodothis vorax (Berk. and Curt.) Sacc. var. *atramentaria* Berk. and Curt., sec. Sacc., 1883, Syll. Fung. 2: 652.

Dothichloë atramentosa Atk., 1905, p. p. Jour. Mycol. 11: 260–261; not *Hypocrea atramentosa* Berk. and Curt., 1869, Cuban Fungi No. 758.

TECHNICAL DESCRIPTION

Hypostromate effuso, tenui, prosenchymatico, albido, in maturitatem nigro epiphyllo, usque 15μ in crassamentum, 200 mm in diam., raro in partibus minoribus diviso. Fructu imperfecto scolecosporo fugaci, in strato effuso ex hypothallo nascente; conidiophoris curtis, simplicibus, raro inflatis et ramosis; conidiis 18μ–30μ longis, 1μ–2μ latis. Fructu imperfecto amerosporo tantum in humore extremo super ascostromatem immaturem patefacto; conidiophoris simplicibus usque spatulatis, omne sterigmate apicali singulum conidium gerente; conidiis obovatis, usque 4μ longis, 3μ latis. Ascostromatibus effusis, maculiformibus, plerumque 10–15 mm in diam., rarius in pulvinulis divisis; superficie plana vel rugulosa, ex ostiolis leviter emersis punctata; contextu albido; peritheciis non arcte densis, ovatis vel lageniformibus, 180μ–315μ longis, 80μ–130μ latis, ostiolis curtis; muris peritheciorum 6μ–12μ crassis; ascis 105μ–160μ longis, 4μ–6μ latis; ascosporis 90μ–120μ longis, 1μ–1.5μ latis.

Hypostroma effuse, as a flat, thin, white prosenchymatous layer or film becoming black with age, on adaxial surfaces of unrolling leaves, rarely exceeding 15μ in thickness (vertical diameter) and 200 mm in horizontal diameter (regarding the leaf surface as the base), rarely as smaller separate units. Scolecosporous conidial fructification ephemeral, white to gray, covering the surface of the hypothallus with a palisade of conidia upon short, simple conidiophores, rarely swollen and branched; conidia 18μ to 30μ by 1μ to 2μ. (When conidia develop on small contiguous hypothalli the conidial fructifications coalesce to form a continuous hymenium.) Amerosporous conidial fructification developing under conditions of extreme moisture upon the immature ascostroma as a layer of simple to spatulate conidiophores, each with an apical sterigma bearing a single conidium; conidia obovate, up to 4μ by 3μ. Ascostromata uniformly effuse, maculiform, usually 10 to 15 mm in horizontal diameter, rarely exceeding 200μ in thickness, sometimes separated into pulvinuli by sterile areas; surface uniform to rugulose, black, punctate from the slightly emergent ostioles; context white; perithecia not crowded, ovate to lageniform, 180μ to 315μ by 80μ to 130μ, ostioles short; perithecial walls 6μ to 12μ thick; asci 105μ to 160μ by 4μ to 6μ; ascospores 90μ to 120μ by 1μ to 1.5μ.

Illustrations: Present paper, pl. 1; Chardon in Mycologia 13, pl. 14, fig. 12; and Diehl in Jour. Agr. Research 41, pl. 2, *D*, *E*, and fig. 3, *B*. The illustrations in the two last-named papers are incorrectly labeled "*Dothichloë atramentosa* Atk."

Distribution: On adaxial leaf surfaces of various grasses from North Dakota and Wisconsin to Florida, and in the West Indies, Mexico, Surinam, and Brazil.

Specimens Cited[4]

On *Calamagrostis canadensis* (Michx.) Beauv.—Wisconsin: 1912, J. J. Davis (N); 1928, J. J. Davis (W); 1919, C. Drechsler (W).

On *C. hyperborea* Lange.—North Dakota: 1922, Brenckle F. Dak. No. 532 (F, W), N. Dak. F. No. 1492 (W).

On *Chloris petraea* Swartz.—Cuba: 1911, Britton and Cowell No. 9899 (N, W). Puerto Rico: 1916, Whetzel and Olive No. 694 (N and in herb. J. A. Stevenson).

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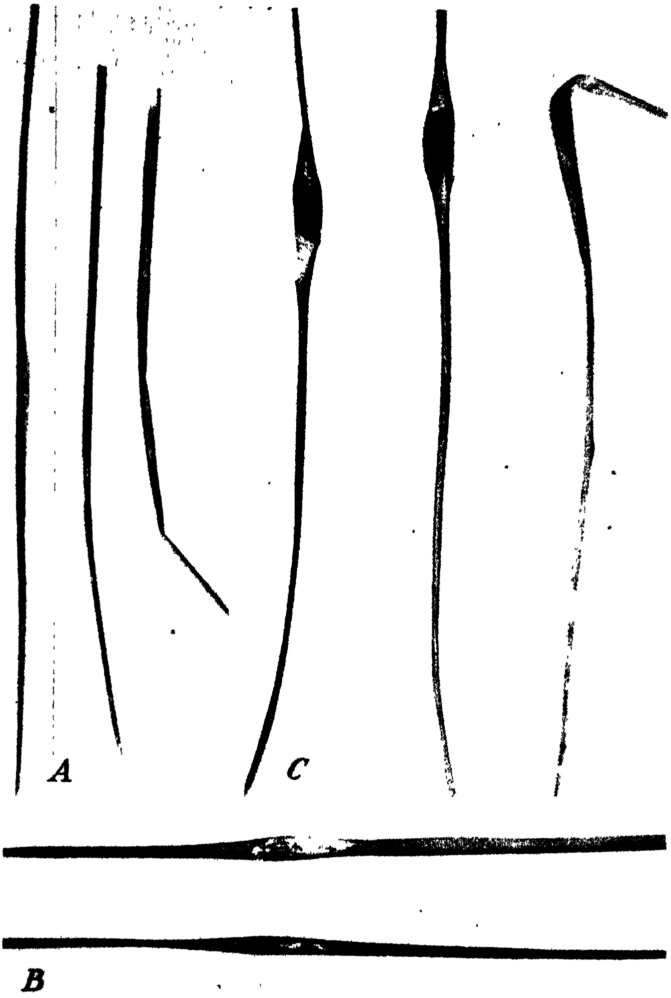
[4] Explanation of abbreviations used in citation of specimens: F = Farlow Herbarium, Cambridge, Mass.; Fl. = herbarium of the Florida Agricultural Experiment Station, Gainesville, Fla.; N = herbarium of the New York Botanical Gardens, New York; W = mycological collections, Division of Mycology and Disease Survey, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

PLATE 1



Leaves of *Sporobolus poiretii* at different stages in the development of *Dothichloë limitata* (× 1): *A*, Unfolding leaves showing constricted region at the point of developing fructifications of *Dothichloe;* B, unfolded leaves showing expanded and rigid regions bearing conidia of the fungus; *C*, leaves bearing the black ascostromata of the fungus.

On *C.* sp.—Mexico: 1910, A S. Hitchcock (W).

On *Ctenium aromaticum* (Walt.) Wood.—North Carolina: 1930, 1931, W. W. Diehl (F, W).

On *Eragrostis capillaris* (L.) Nees.—South Carolina: 1876, H. W. Ravenel in DeThüm. Myc. Univ. No. 1068 as *Dothidea atramentaria* B. and C. f. *eragrostidis capillaris* (F, W, N). (Paratype of *Dothichloe limitata.*) (The host here may very well be *E. refracta.*)

On *E. hirsuta* (Michx) Nees.—Georgia: 1933, J H. Miller (W).

On *E. refracta* (Muhl.) Scribn.—Georgia: 1931, W. W. Diehl (F, W). Florida: 1931, Weber, West., and Diehl (F, W). Virginia: 1932, W. W. Diehl (F, W).

On *E.* (?) sp.—South Carolina: ("Gramineae" of label) H. W. Ravenel No. 259 in Herb. Curtis (F); ('*Poa*' of label) Ravenel, F. Am. Exs. No. 100 (F, W, N); Ellis N. Am. F. No. 683 (F, W, N). Florida: 1897, R. Thaxter in herb. Thaxter (F).

On *Gymnopogon ambiguus* (Michx.) B. S. P.—Virginia: 1936, R. F. Smart (W).

On *Panicum* sp.—Mississippi; 1920, L. E. Miles 323 (W).

On *Panicum agrostoides* Spreng.—Missouri: 1906, H. L. Shantz (W).

On *Panicum* sp.—Mississippi: 1920, L. E. Miles No. 323 (W).

On *Sporobolus poiretii* (Roem. and Schult.) Hitchc.—Louisiana: 1931, R. W. Davidson (F, W). Mississippi: 1922, L. E. Miles No. 324 (W); 1930, R. J. Haskell (F, W). Alabama: 1898 (on "*Sporobolus radicans*" of label), G. W. Carver (Fl.); Auburn, July 17, 1929, J. L Seal, type (F, W); 1931, J. L. Seal and W. W. Diehl (F, W). Georgia: 1908, H. A. Allard (W); 1930, 1931, J. B. Demaree (W), 1931 W. W. Diehl (F, W); 1932, J. E. Benedict, Jr (F, W); 1933, Geo Crisfield (F, W). Florida: 1928, G. F. Weber and ——— Nolan (Fl.); 1931, W. W Diehl (F, W). Costa Rica: 1923, F. L. Stevens Nos. 10, 111, 151, 157 (W). Brazil: (Minas Geraes) 1929, Agnes Chase No 10199½ (W).

On *Thrasya petrosa* (Trin.) Chase—Brazil: 1930, A. Chase No. 11287 (W).

On Gramineae.—Surinam: 1827, Weigelt as *Sphaeria epichloe* Kze. (W).

IDENTITY OF SYNONYMS

It is possibly needful to explain here the particular reasons for proposing a new name to replace the early specific name "*epichloë*" and the later and much garbled "*atramentaria.*"

In the first place, it is questionable whether any of Weigelt's specific names possess validity unless published elsewhere than on the unnumbered specimens distributed by Kunze.[5] Any possible standing this specific name might have had is seriously brought into question by its particular validation by Saccardo,[6] who after studying the specimen of *Sphaeria epichloë* Kze. from the Royal Herbarium at Brussels described the material under the name *Botryosphaeria* (?) *epichloë* (Kze.) Sacc. He did not observe asci and noted spores "$7\mu \times 4\mu$," which he suggested might be conidia of *Dothiorella*; and it would seem to be a conidial fungus of questionable identity. Examination of the Weigelt specimen in the mycological collections of the Bureau of Plant Industry reveals it to be *Dothichloë limitata* without any evidence of contaminating *Dothiorella* or *Botryosphaeria.* It thus seems preferable to restrict Saccardo's name and description to some immature fungus not adequately described and not really significant in the nomenclature of the species under consideration, and to regard Kunze's name as invalid.

The name "*atramentaria*" used as a nomen nudum on the several fungi exsiccati cited above was apparently merely an error in spelling or printing for *atramentosa.* There is every reason to assume that Berkeley in 1876 [7] and Saccardo in 1883,[8] in publishing the name without a description, as well as Ravenel, DeThümen, and Ellis, in their use of the name on exsiccati, indicated no new species. Subsequent students of these two species have seemingly given this little or no concern. Atkinson[9] used the name *Hypocrea atramentosa* Berk. and Curt., of Berkeley's Cuban Fungi,[10] as the basis for *Dothichloë atramentosa* (Berk. and Curt.) Atk., although with apparent misgivings he included in his concept the species here called *D. limitata*. Atkinson clearly distinguished his species by its orientation upon lower leaf surfaces, but because of the "very thin stroma" expressed his doubt about the fungi exsiccati labeled "*Dothidea atramentaria*." Most curiously, he did not note the limitation of this "very thin stroma" to the upper or adaxial leaf surfaces. Both species have been grown by the writer contemporaneously, although upon different hosts, and their specific peculiarities in orientation have remained constant, i. e., *Dothichloè atramentosa* remained abaxial, and *D. limitata* adaxial. Differences in appearance and orientation might be explained as reactions owing to particular hosts were it not for the fact that, even when occurring on the same host species, the two fungi exhibit these differences in orientation. Both have been found and can be readily identified upon three grasses: *Chloris* sp., *Gymnopogon ambiguus*, and *Panicum agrostoides*.

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[5] Specimens gathered by C. Weigelt in Surinam were named and distributed by Gustav Kunze in 1828 or thereabouts, bearing a printed label but lacking any numerical basis for citation The particular printed label in question is as follows. "*Sphaeria* (periph pulvin) *epichloë* Kz S (epiphylla hypogena) oblonga gemella, atra: peritheciis ovalibus, ostiolo papillato, demum pertuso In foliis gramineae, Gujan, Surinam, leg et exsicc. Weigelt 1827. Determ. Kunze "

[6] SACCARDO, P. A. FUNGILLI ALIQUOT HERBARII REGNI BRUXELLENSIS. Compt Rend. Soc. Roy. Bot. Belg. 31 (pt 2): 224-258. 1892.

[7] BERKELEY, M. J NOTICES OF NORTH AMERICAN FUNGI NO 886 Grevillea 4. 105 1876.

[8] SACCARDO, P. A. SYLLOGE FUNGORUM . . . v. 2, p. 814. 1883. Padova.

PATHOLOGICAL CONSIDERATIONS

IDENTITY OF HOST SPECIES

The list of host names for specimens cited, included in the section on Taxonomy, shows a wide range of grass genera but does not pretend to comprise all susceptible species, because no extensive field studies have ever been undertaken. But where a specific name is noted there is reasonable certainty of identity. Through the kindness of Mrs. Agnes Chase and the late A. S. Hitchcock, reidentification or verification of particular specimens has been possible. The observer in the field is usually able to note the similarity of diseased (i. e. sterile but healthy-appearing) grass plants to nearby normal inflorescence-bearing specimens; verification of the then-assumed specific identity is readily made later in the laboratory by microscopic comparison of leaf sections.

FIELD OBSERVATIONS

Although adequate field studies have not been possible, it seems pertinent to note the localities and dates of field observations of diseased plants because they show some agreement with records of diseased plants cultivated under relatively continuous scrutiny in the greenhouse and garden. The following are the author's personal records of host, locality, month, and year for diseased plants noted, except that when later visits to the same place failed to detect the affected plants such dates are indicated by an asterisk (*):

On *Ctenium aromaticum:* Near Burgaw, N. C., April 1930, August* 1931, September 1931, December 1932, June 1933, September 1934, April 1938.

On *Eragrostis refracta:* Near Gainesville, Fla., September 1931; near Thomasville, Ga., September 1931; near Petersburg, Va., September 1932, September* 1933, October 1938.

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[9] ATKINSON, G. F. THE GENERA BALANSIA AND DOTHICHLOË IN THE UNITED STATES WITH A CONSIDERATION OF THEIR ECONOMIC IMPORTANCE. Jour. Mycol. 11: 248-267, illus. 1905.

[10] BERKELEY, M. J. ON A COLLECTION OF FUNGI FROM CUBA. PART II., INCLUDING THOSE BELONGING TO THE FAMILIES GASTEROMYCETES, CONIOMYCETES, HYPHOMYCETES, PHYSOMYCETES, AND ASCOMYCETES. Jour. Linn. Soc. [London], Bot. 10: 341-392. 1869.

On *Sporobolus poiretii* Auburn, Ala., September 1931; near Thomasville, Ga., 1931; Chattahoochee, Madison, and Gainesville, Fla., September 1931; Burgaw, N. C., April* 1931, September 1931, December* 1932, June 1933, September 1934, April* 1938.

At each time noted by an asterisk (*), considerable effort was made to find specimens, but without success. In these cases, however, the failure is explained by such local conditions as drought, destruction by fire or other causes, or by the dormant season. When growth was lush, the fungus was abundantly in evidence on certain plants. On these plants most leaves bore the fungus or showed certain chlorotic areas, which, as will be described later, are characteristic of dormancy or retardation of the fungus. With these slight exceptions, there was no difference between the color of diseased plants and that of healthy ones; diseased plants, even when abundant, are thus usually difficult to distinguish, because the mere absence of inflorescences in certain ones among thousands easily passes unnoticed. The limitation of infection to certain plants, on which practically every leaf bore the fungus while neighboring plants remained free from infection, suggested either marked differences in susceptibility of the several plants or else systemic infection. The latter hypothesis seemed the more reasonable in view of a lack of plants showing any intermediate condition. These observations were supplemented by the study of infected plants under garden and greenhouse culture where conditions permitted some degree of experimental control.

GARDEN AND GREENHOUSE CULTURES

Outdoor Transplantings

Infected plants (clumps) of three host species, namely, *Ctenium aromaticum*, *Sporobolus poiretii*, and *Eragrostis refracta*, have at various times been transplanted to a garden in Arlington, Va., and kept under observation particularly with regard to the succession of fungus fructifications and the possible development of normal inflorescences upon the plants. In each case the fungus-bearing leaf blades were removed, compelling the forcing of new leaves.

Table 1.—*Summary of garden transplantings of grasses infected with Dothichloë limitata*

Host	Origin	Time of transplanting	Time of last observation	Infected plants surviving
				Number
Ctenium	Burgaw, N. C.	April 1930	September 1930	5
		September 1931	October 1931	3
		April 1938	September 1938	2
Eragrostis	Gainesville, Fla.	September 1931	October 1931	4
	Petersburg, Va.	August 1932	October 1934	2
Sporobolus	Gainesville, Fla.	September 1931	October 1931	6
	Auburn, Ala.	July 1929	October 1929	6
		September 1931	October 1931	2
	Bogalusa, La.	July 1931	November 1932	4

During the months of June and July 1930, no observations were made, and during the season of June to September 1931, inclusive, 5 clumps of this origin were likewise grown in the open at the Harvard Botanical Garden, Cambridge, Mass., although inspected but once in August.

Except for the plantings made in September (table 1), in each case a healthy clump of a grass was planted contiguous to each diseased clump of the same species, so that the new leaves on the healthy plants might become infected upon coming in contact with the fungus on the leaves of the adjacent diseased plants. These healthy plants, however, remained free of any infection during the entire time, whereas the newly forced leaves on the diseased transplants bore the fungus in regular succession until late autumn, when the newer leaves ceased to bear fungus fructifications.

Although healthy transplants of *Ctenium* remained sterile, those of *Eragrostis* in 1933 and 1934 and of *Sporobolus* in 1929 produced normal inflorescences. In contrast, none of the diseased plants developed any inflorescences or peduncles. In connection with the following studies of diseased plants under greenhouse conditions, additional observations on these outdoor plantings are compared with the results of the more controlled greenhouse cultures.

Greenhouse Cultures

In the autumn of 1930 the 3 above-noted infected grass species were removed to a greenhouse in the Harvard Botanical Garden at Cambridge, Mass., but only the *Ctenium* survived. There were 10 of these successful transplantings, in 10-inch pots. Since the greenhouse proved too cool in late autumn for rapid growth, 5 of the transplants were removed to a much warmer greenhouse available at the substation of the Massachusetts Agricultural Experiment Station at Waltham, where they were kept from late November through the month of February 1931, after which they were returned to the cooler Harvard greenhouse. In May 1931, when the Harvard greenhouse was warmer, the new *Ctenium* leaves again produced characteristic *Dothichloe* fructifications.

The Waltham greenhouse temperature averaged about 23° C., running considerably higher on sunny days but rarely dropping below 20° at night, so that the greenhouse at Waltham may be termed the warm greenhouse, while the one at Cambridge, where nighttime winter temperatures occasionally reached a minimum of 7°, may be termed the cool greenhouse. As a further check, two clumps transplanted to 6-inch pots were kept, from December 28 to April 6, under close observation in a Wardian case in the laboratory, where during that period the temperature normally ranged from 20° to 27°, with but seven instances of a 15° minimum.

During the entire time the plants were in the cool greenhouse, November to April, inclusive, there was very little vegetative growth of the *Ctenium* and no new appearance of the fungus. On the other hand, in the warm greenhouse the *Ctenium* plants grew new leaves at a rate comparable to summertime growth in the garden, and the fungus fructifications appeared on each leaf. Likewise, on the two plants kept in the Wardian case in the laboratory the fungus developed fruits upon the new leaves. Although in the cool greenhouse no new *Ctenium* leaves bore any fungus fructifications, they tended to show, but not uniformly, certain faintly chlorotic bands, occasionally with some constriction suggestive of the chlorotic areas where the fungus appeared normally. This same phenomenon was noted regularly during cool weather in the field and in garden plantings. Microscopic examination of such tissues, and of normal green-leaf tissues of infected plants as well, revealed the minute hyphae, 1μ to 2μ in diameter, characteristic of *Dothichloë* infection

These observations on the behavior of the fungus in infected plants under low and normal temperatures indicate that low temperatures, although high enough for vegetative growth of both host and parasite, inhibit the fructifications of the fungus. It has been shown that a

5-month suppression of fructifications in the cool greenhouse had no inhibiting effect upon subsequent spore production after the infected grasses were again subjected to normal growing temperatures. The continued production of infected leaves without spore production through a long period also bore out the supposition that the infection was systemic.

In this connection it is noteworthy that in the field, as well as in garden and greenhouse culture, even under normally high-temperature conditions, infected plants of *Ctenium* occasionally bear some leaves that show neither chlorotic bands nor fungus fructifications, although microscopic examination of such leaves has revealed characteristic infecting hyphae.

INOCULATIONS

The presence on infected plants of seemingly uninfected leaves with or without slightly chlorotic bands was at first thought to be evidence that *Dothichloe limitata* caused localized disease areas rather than systemic infection. With that assumption, numerous attempts were made in greenhouses in Massachusetts, at Cambridge and at Waltham, during the winter season of 1930–31, and in garden culture at Arlington, Va., during the seasons of 1929, 1931, 1932, and 1934, to secure inoculation of emerging leaves on plants of *Ctenium aromaticum* and of 30 other possibly susceptible grasses, but without success. The methods used, in addition to the interplanting of healthy and diseased plants previously noted, were standard practices: Atomizer spraying and application of spores by both brush and fingertip rubbing, as well as needle injection by means of a hypodermic syringe, with germinable and germinating scolecosporous conidia and in part with ascospores. The complete failure to produce the disease by the inoculation of growing tissue in mature plants tended to discount the assumption of localized infection, yet it in nowise conflicts with the likelihood of systemic infection.

HOST SYMPTOMS AND SUPPRESSION OF FUNGUS FRUCTIFICATIONS

In greenhouse and garden, as in the field, newly forced leaves of infected plants tended to remain inrolled and at certain points on the blade failed to expand at the same rate as other leaves (pl. 1, *A*). At these points, on all hosts noted, there was a slight chlorosis, the constricted region being paler than the rest of the leaf. The whitish conidial fructifications were borne upon the adaxial leaf surfaces in the constricted region, maturing fully before this constricted region expanded (pl. 1, *A* and *B*). When these spore-bearing surfaces were fully expanded they became rigid and were chiefly noticeable because apparently unable to roll up to the same extent as the rest of the leaf on drying. In most cases, after the conidial fructifications reached maturity they were succeeded in from 1 to 2 weeks by black ascostromata (pl. 1, *C*) bearing perithecia and ripe ascospores. These ascostromata disintegrated shortly after reaching maturity. After the rather evanescent ascostromata disappeared, the stiffened leaf areas remained rigid and slightly chlorotic, tending to form a conspicuously expanded area on a normally inrolling recurving leaf. These leaves never seemed otherwise to suffer any immediate disadvantage, because they remained a nearly normal green color long after the stromata had disappeared. In all cases under observation, however, individual plants so infected have never shown any tendency to produce inflorescence. This coincides with field records in that inflorescences and seed production appeared to be absent uniformly from plants infected with *Dothichloë limitata.*

In garden culture of infected plants it has been noted consistently that on the newer leaves produced in late autumn no fungus fructifications developed, the leaves being seemingly free of infection. But in *Ctenium* there were slightly chlorotic leaf areas comparable in size and position to fungus-bearing areas, although they remained sterile. Microscopic examination, however, always revealed the characteristic mycelium 1μ to 2μ in diameter, in such tissues.[11] This chlorotic tendency was less noticeable in *Sporobolus* and in *Eragrostis* than in *Ctenium.*

DISCUSSION

The host plants thus far recorded for *Dothichloë limitata* are widely distributed geographically, although except for the two species of *Calamagrostis* they are not of economic importance. It is, however, of significance that when infected by this fungus these various grasses are sterilized by it. This condition of sterility generally passes unnoticed because it lacks the conspicuous chlorotic effects or obvious lesions usually associated with disease. The parasite, which sterilizes the host through preventing the formation of any semblance of an inflorescence but which produces no other readily recognizable effects, presents a role that has been little considered in phytopathological literature. It it is not convenient here to attempt an evaluation of the possible factors affecting this sterility phenomenon, but such incidental observations as those upon the effect of low temperature in preventing sporulation of the parasite may well aid in explaining its predominantly southern range. In more northern regions the fungus may be present in grass tissues without sporulation and therefore without observational record. In those cases its presence could be detected only by microscopic examination. The presence of minute hyphae in seemingly healthy grass tissues has long been known, particularly in the case of various inconsistent records of observations and discussions of "the *Lolium* fungus." *Dothichloë limitata* is doubtless merely one of a number of similar intramatrical parasites of grasses and sedges that sterilize their host plants without producing any conspicuous lesions or chlorosis.

SUMMARY

Dothichloë limitata, here described as new, is distinguished from a closely related fungus by its morphology and its habits.

Its known distribution, from Brazil through the southeastern part of the United States, with a host range comprising species of eight different grass genera, is based on an extensive herbarium record.

Observations of infected grasses in field and greenhouse indicate that infection is systemic.

It is shown that low temperatures inhibit the fructifications of the fungus but do not eliminate the infection.

Infected plants, except for a failure to produce inflorescences, appear superficially healthy because of a normal coloration.

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[11] Such hyphae are demonstratable by crushing and macerating the mycelium under a cover glass and then staining it with erythrosin (phloxine).

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